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GROWTH OF FRUITING PARTS IN GOSSYPIUM CERNUUM, AN ASIATIC COTTON[1]

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INTRODUCTION

Studies on the growth of the fruiting parts of several varieties of American upland cotton (*Gossypium hirsutum*) and on Pima Egyptian and sea-island cotton (*G. barbadense*) have been reported in recent publications of the United States Department of Agriculture.[2] Although comparisons were made under a rather wide range of conditions, only slight differences were found in the rate of growth of the fruiting parts of the two types. Since the Asiatic cottons are in many respects widely different from both upland and sea-island, it seemed worth while to extend the comparison to include one of these.

Gossypium cernuum (Tod.), commonly known as Garo Hill or wool cotton, a native of India, is a species with so many divergent characters that differences in its habits of growth might reasonably be expected. Accordingly, a study of the growth of the fruiting parts of this species was begun at Greenville, Tex., in 1924. Data were recorded on the succession of fruiting branches, period of bud development, succession and growth measurements of floral buds, shedding of buds and young bolls, and growth measurements and maturation period of bolls. Comparable data were recorded for the Lone Star variety of upland cotton grown under the same conditions.

While the results of these studies indicate that Garo Hill differs but little in its growth periods from the American upland varieties or the American Egyptian and the sea-island varieties, as previously reported, the data are of interest as a further contribution to the available information on the fruiting habits of the cotton plant in relation to production under boll-weevil conditions. Some of the

[1] Received for publication Sept. 1, 1926; issued August, 1927.

[2] BALLARD, W. W., and SIMPSON, D. M. BEHAVIOR OF COTTON PLANTED AT DIFFERENT DATES IN WEEVIL-CONTROL EXPERIMENTS IN TEXAS AND SOUTH CAROLINA. U. S. Dept. Agr. Bul. 1320, 44 p., illus. 1925.
MCNAMARA, H. C., HUBBARD, J. W., and BECKETT, R. E. GROWTH AND DEVELOPMENT OF COTTON PLANTS AT GREENVILLE, TEX. U. S. Dept. Agr. Circ. 401, 18 p. 1927.
MARTIN R. D., BALLARD, W. W., and SIMPSON, D. M. GROWTH OF FRUITING PARTS IN COTTON PLANTS. Jour. Agr. Research 25: 195–208, illus. 1923.

Asiatic cottons are adapted to short-season conditions, but the bolls are too small for economical picking by hand. The bolls of the Garo Hill are the largest of the Asiatic series, and are of a size comparable with small-bolled upland varieties.

SUCCESSION OF FRUITING BRANCHES

The appearance of the fruiting branches on the cotton plant has been described as follows: "The first indication of a new fruiting branch is the appearance of a minute triangular bud, commonly called a 'square,' deeply inclosed between the stipules of the primary leaf. The appearance of the square always precedes the development of the internode on which it is borne, and may therefore be considered as a definite indication of the formation of a fruiting branch." [3] This criterion was used in recording the appearance of fruiting branches on Garo Hill.

The interval between the appearance of successive fruiting branches on the main stalk was variable, ranging from 1 to 2 days early in the season, when the plants were making a rapid growth, to 8 or 9 days late in the season when plant growth was much slower. The mean interval was $2.31 \pm .177$ days.

TABLE 1.—*Interval between the appearance of successive fruiting branches on Garo Hill, Lone Star, and Pima cotton*

Variety	Locality	Year	Mean number of days between appearance of successive fruiting branches
Garo Hill	Greenville, Tex	1924	$2.31 \pm .177$
Lone Star	do	1924	$2.84 \pm .077$
Pima	Sacaton, Ariz	1921	$2.81 \pm .043$

From the figures presented in Table 1, it can be seen that the mean interval between the appearance of successive fruiting branches produced on the main stalk of Garo Hill was about one-half day shorter than that recorded on Lone Star and Pima, but the probable error of the difference shows that it is not significant.

SUCCESSION OF FLORAL BUDS

The date that each square appeared on the fruiting branch was recorded, and from these records it is possible to compute the number of days between the appearance of successive squares or buds on the fruiting branches. This interval was found to range from 3 to 12 days, with a mean of $6.50 \pm .167$ days. Of the 196 cases recorded only 3 had an interval of less than 4 days and 8 over 10 days. There was no significant difference in this interval between Garo Hill and Lone Star or Pima. The mean number of days between the appearance of successive squares [4] on the fruiting branches of Garo Hill, Lone Star, and Pima are given in Table 2.

[3] MARTIN, R. D., BALLARD, W. W., and SIMPSON, D. M. GROWTH OF FRUITING PARTS IN COTTON PLANTS. Jour. Agr. Research 25: 195–208, illus. 1923.

[4] The term "appearance of square" is used to designate the time when the triangular fruiting bud becomes visible to the naked eye.

TABLE 2.—*Mean number of days between the appearance of successive squares on the fruiting branches of Garo Hill, Lone Star, Pima cotton*

Variety	Locality	Year	Mean number of days between appearance of successive squares on fruiting branches
Garo Hill	Greenville, Tex	1924	6.50±.167
Lone Star	do	1924	6.11±.107
Pima	Sacaton, Ariz	1921	6.35±.069

A tendency was noted in Garo Hill for the interval between appearance of successive squares on the fruiting branches to lengthen when plant growth was retarded late in the growing season. This is in agreement with results reported on other species of cotton.

GROWTH MEASUREMENTS OF FLORAL BUDS

Daily growth of the floral buds was recorded by measuring the length from the base of the calyx to the corolla tip. The measurements were begun when the buds had been visible from 7 to 8 days, at which time they were 2 to 3 mm. There was a mean interval of 18 days from the date of first measurement until bloom, with only 10 deviations from the mean. The mean daily growth measurements of the floral buds on Garo Hill, together with those recorded on Lone Star in 1922,[5] are given in Table 3.

TABLE 3.—*Mean length of floral buds of Garo Hill and Lone Star from 18 days to 1 day preceding bloom*

Days preceding bloom	Mean length of floral buds of—		Days preceding bloom	Mean length of floral buds of—	
	Garo Hill, 1924	Lone Star, 1922		Garo Hill, 1924	Lone Star, 1922
	Mm.	*Mm.*		*Mm.*	*Mm.*
18	2.13		9	9.62	9.67
17	2.94		8	10.47	10.30
16	3.91		7	11.34	11.17
15	4.81	6.02	6	12.00	12.22
14	5.72	6.57	5	13.20	13.10
13	6.48	7.20	4	14.69	14.28
12	7.37	7.62	3	16.15	15.60
11	8.05	8.36	2	19.10	17.70
10	8.78	8.96	1	29.80	24.37

From these data it can be seen that the elongation of the average Garo Hill floral bud for the first 13 days on which measurements were made was about 0.8 mm. per day. During the next three days the increase was approximately 1.3 mm. per day. The second day preceding bloom the average elongation was 2.93 mm. The mean length of the floral bud on the day before bloom was 29.8 mm., or an increase of 10.7 mm. over the length on the second day before bloom.

[5] MARTIN, R. D., BALLARD, W. W., and SIMPSON, D. M. Op. cit.

This marked increase in the length of the floral bud on the day preceding bloom is due to the rapid enlargement of the corolla and inclosed staminal column.

Fifteen days before blooming the Garo Hill floral buds were somewhat smaller than those of Lone Star, but the daily increase in length was slightly more rapid than in Lone Star, so that by the ninth day

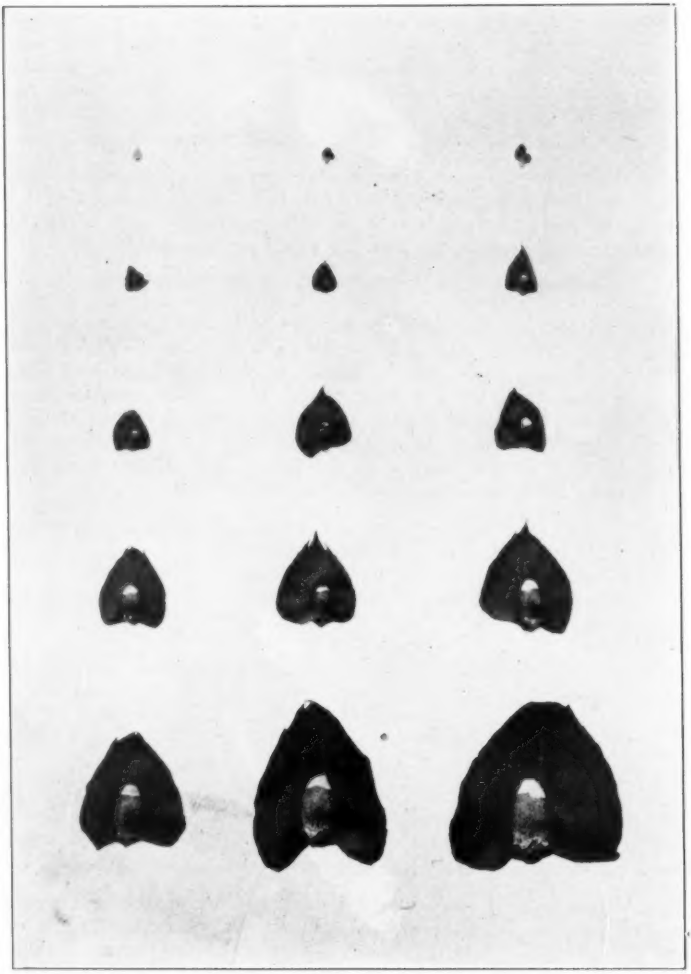

FIG. 1.—Floral buds of Garo Hill cotton (*Gossypium cernuum*), Greenville, Tex., 1924, showing the size of the floral buds from 23 to 9 days preceding bloom. Natural size

preceding bloom the buds were nearly equal in length. On the second day previous to bloom the length of the average Garo Hill floral bud was 19.1 mm., and Lone Star was 17.7 mm. The day before flowering the Garo Hill buds increased 10.7 mm., and the

Lone Star buds 6.67 mm., so that the final mean lengths were 29.8 for Garo Hill and 24.37 mm. for Lone Star.

The daily growth of the floral bud of Garo Hill is illustrated in

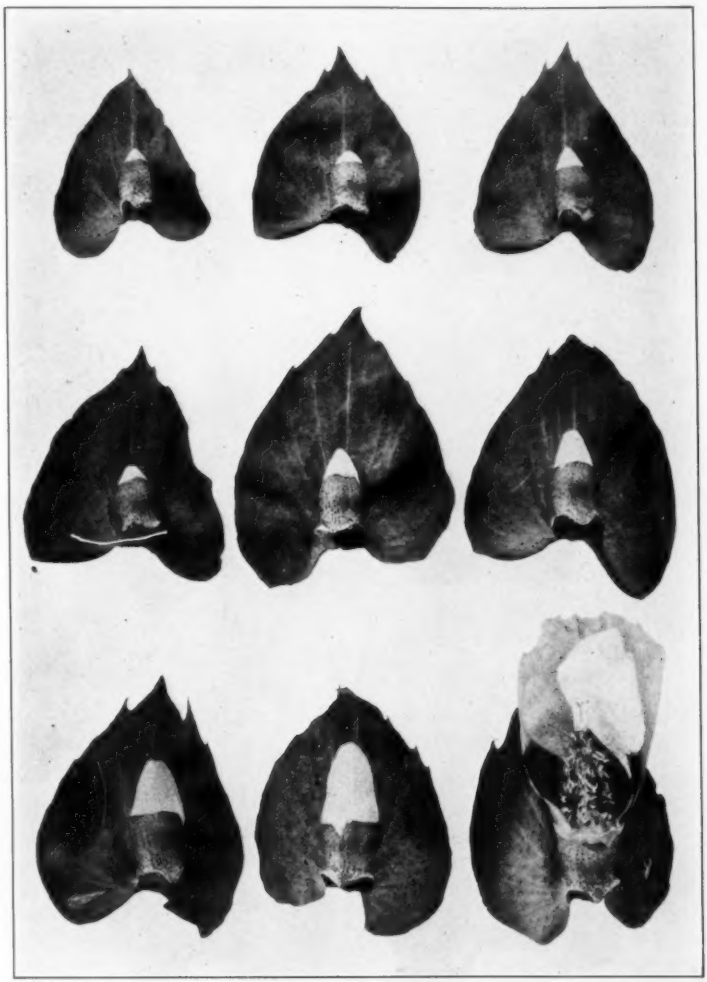

FIG. 2.—Floral buds of Garo Hill cotton (*Gossypium cernuum*), Greenville, Tex., 1924, showing the size of the floral buds from eight days preceding bloom until bloom. $\times\frac{4}{15}$

Figures 1 and 2, which together show the size of the floral bud and one bract daily for 23 days preceding bloom. Figure 3 shows the size at 3-day intervals.

PERIOD OF BUD DEVELOPMENT

The date that each bud appeared and also the date that each bud flowered were recorded, and from these dates the square period [6] was

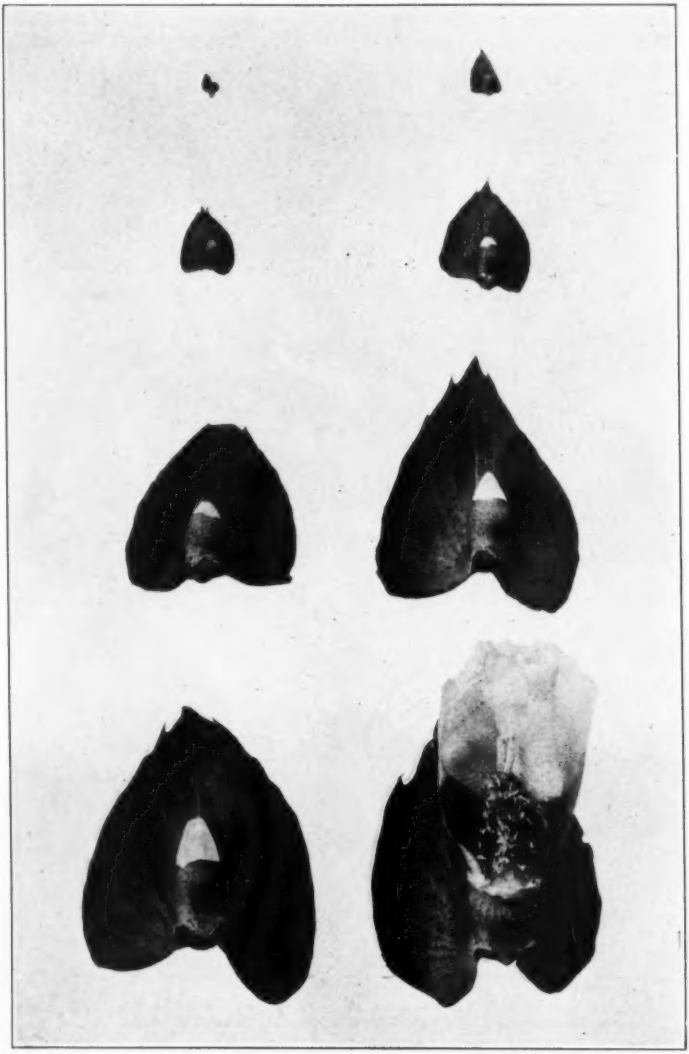

FIG. 3.—Floral buds of Garo Hill cotton (*Gossypium cernuum*), Greenville, Tex., 1924, showing the size of the floral buds at three-day intervals from 21 days preceding bloom until bloom. Natural size

determined. This period varied from 22 to 30 days, with a mean of 25.64 days. Buds produced late in the season, when plant growth

[6] The number of days from appearance of the bud until it flowers is referred to as the "square period."

had practically ceased, required a longer period than those produced earlier in the season. Only 4 of the 205 squares examined had a square period of 30 days, and 3 of these were on the topmost fruiting branches, while the fourth was on the outer node of a lower branch.

The mean square period for Garo Hill was 25.64 ± .32 days, which was approximately the same as that recorded for Lone Star, namely, 25.93 ± .10. The square period recorded for Pima in 1921 was 30.11 ± .128. From these figures it is evident that the square period for Garo Hill and Lone Star was about 4.5 days less than for Pima.

SHEDDING OF BUDS AND YOUNG BOLLS

The interval from the appearance of buds to the date of shedding was very irregular both in Garo Hill and Lone Star, ranging from 2 to 18 days, with a mean of 10.5 ± .669 days for Garo Hill and 8.28 ± .467 days for Lone Star. There was a tendency in both cottons for the buds produced early in the season to shed in fewer days than those produced late in the season.

The number of days from flowering to shedding of bolls was very regular in Garo Hill, varying from 2 to 5, with a mean of 3.85. This period ranged from 1 to 11 days in Lone Star, with a mean of 6.10 days. These ranges are shown in Table 4, which gives the frequency distribution of the number of days from flowering to shedding of bolls for Garo Hill and Lone Star.

TABLE 4.—*Frequency distributions of the number of days from flowering to shedding of bolls on Garo Hill and Lone Star in 1924*

Variety	Number of bolls shed from 1 to 11 days after flowering, as indicated											Mean number of days
	1	2	3	4	5	6	7	8	9	10	11	
Garo Hill		2	25	35	17							3.85
Lone Star	1	1	4	10	15	20	18	11	4	2	1	6.10

The mean number of days from flowering to shedding of bolls was 3.85 ± .059 days for Garo Hill. This was 2.26 ± .06 days less than recorded for Lone Star, which was 6.10 ± .01 days. Martin and Loomis [7] report that the mean persistence of shed bolls in Pima at Sacaton, Ariz., in 1920 was 10.8 days. This is about 7 days longer than that recorded for Garo Hill.

Whether it is a normal occurrence for the Garo Hill bolls to shed so soon after flowering or whether it was due to traumatic causes has not yet been determined. All of the bolls shed from the Garo Hill plants studied were carefully examined for insect injuries, and only five of the total shed appeared to be injured.

GROWTH MEASUREMENTS OF BOLLS

Data on the growth of the bolls were obtained by measuring the length, beginning the second day after flowering and continuing

[7] MARTIN, R. D., and LOOMIS, H. F. SUMMER IRRIGATION OF PIMA COTTON. Jour. Agr. Research 23: 927-946, illus. 1923.

daily for 20 days, at which time the bolls had attained their maximum length. The following record is based on measurements obtained on 176 bolls that reached maturity. The mean length of the bolls on the second day after flowering was 14.09 mm., and the mean daily increase in length was about 1.5 mm. for the next four days. Thereafter the growth rate was approximately 2.2 mm. per day until the bolls were 12 days old and had reached a mean length of 33.55 mm. The rate of growth after this age was attained was considerably slower, the bolls reaching a mean length of 37.67 mm. eight days later, which was an increase of only half a millimeter per day.

The growth rate on the Garo Hill bolls was similar to that recorded on Lone Star bolls at Greenville, Tex., in 1922.[8] The daily growth measurements on Garo Hill bolls are shown in Table 5.

TABLE 5.—*Mean length of bolls of Garo Hill from 2 to 20 days after flowering*

Days after flowering	Length of bolls	Days after flowering	Length of bolls
	Mm.		*Mm.*
2	14.09±0.178	12	33.55±0.276
3	15.73±0.198	13	35.44±0.255
4	17.18±0.292	14	36.52±0.278
5	18.36±0.265	15	37.05±0.247
6	20.57±0.264	16	37.35±0.264
7	23.00±0.249	17	37.51±0.356
8	24.50±0.320	18	37.63±0.291
9	27.15±0.295	19	37.67±0.306
10	29.33±0.212	20	37.67±0.306
11	31.65±0.400		

The increase in size of the bolls is illustrated in Figure 4, which shows the size of the bolls every 5 days from bloom until 25 days after.

MATURATION PERIOD OF BOLLS

The period of maturation of Garo Hill bolls was found to vary from 39 days for bolls set early in August to 56 days for those set in September. The maturation period of bolls set at four 6-day periods from August 9 to September 1, is shown in Table 6. The lengthening of this period as the cotton plants reached their full growth was also found in Lone Star at Greenville, Tex., in 1924, which varied from 37.2 days for bolls set early in July to 53.7 days for those set in August. King[9] reports that the maturation period of Pima bolls at Phoenix, Ariz., in 1919, varied from 54 days for bolls set in July to 80 days for those set in September.

TABLE 6.—*Maturation period of bolls of Garo Hill set at four 6-day intervals from August 9 to September 1, 1924*

Maturation period of bolls set—	Days
August 9–14	40.67
August 15–20	44.31
August 21–26	50.78
August 27–September 1	52.60

[8] MARTIN, R. D., BALLARD, W. W., and SIMPSON, D. M. Op. cit.

[9] KING, C. J. WATER-STRESS BEHAVIOR OF PIMA COTTON IN ARIZONA. U. S. Dept. Agr. Bul. 1018, 24 p., illus. 1922.

The mean maturation period of all Garo Hill bolls set was 45.49 ± .271 days. This was 4.66 days longer than that recorded for Lone Star in 1924 and 22.51 days shorter than that recorded for Pima.[10]

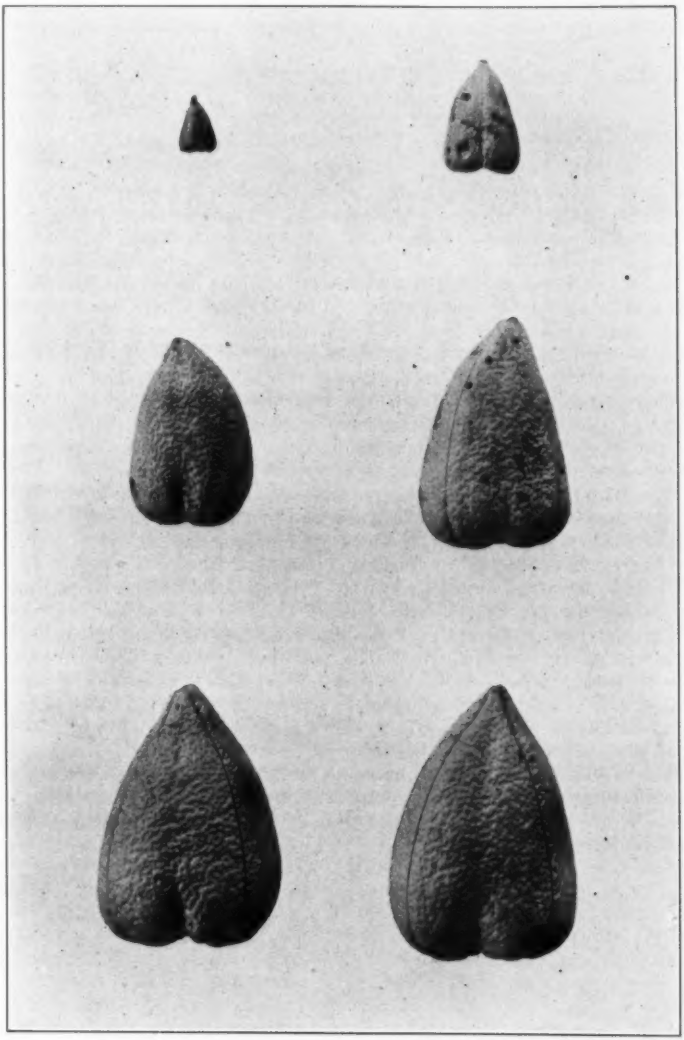

FIG. 4.—Bolls of Garo Hill cotton (*Gossypium cernuum*), Greenville, Tex., 1924, showing sizes of bolls at five-day intervals, from the flower to the adult size at 25 days. Natural size

The mean maturation period of Garo Hill, Lone Star, and Pima is presented in Table 7.

[10] KING, C. J. Op. cit.

TABLE 7.—*Mean maturation period of bolls of Garo Hill, Lone Star, and Pima*

Variety	Locality	Year	Mean maturation period
			Days
Garo Hill	Greenville, Tex.	1924	45.49±.271
Lone Star	do	1924	40.83±.178
Pima [a]	Phoenix, Ariz.	1919	68.00

[a] KING, C. J. Op. cit.

SUMMARY

The Garo Hill cotton (*Gossypium cernuum*) is one of the Asiatic series which is very remote in plant characters from the Egyptian and upland varieties grown in the United States, so that differences in habits of growth might be expected. Most of the Asiatic cottons are not adapted for cultivation in the United States on account of their very small bolls, but the Garo Hill has the largest bolls of any Asiatic type; nearly as large as those of the Texas big-boll cottons, as represented in this comparison by the Lone Star variety.

The growth rates and sequence in development of the fruiting parts of the Garo Hill cotton were determined at Greenville, Tex., by the same methods that were used on the Egyptian and upland types of cotton, and reported in previous publications.

The interval between the appearance of successive fruiting branches on the main stalk is somewhat shorter in the Garo Hill cotton than in the other types, while the interval between successive squares on the branches is somewhat longer. The shedding of abortive bolls in Garo Hill occurred more promptly, within 3 to 5 days after flowering, while many abortive bolls of Lone Star were held for 6 to 8 days, and some from 9 to 11 days. The rate of growth of the buds and bolls was nearly the same in the Garo Hill cotton as in the other types. The maturation period of bolls of the Garo Hill was somewhat longer than that of Lone Star, though the difference was slight and may not be significant.

In the latter part of the season a lengthening of the maturation period of Garo Hill bolls was found, which also is in agreement with the behavior of the Lone Star and Pima varieties. Garo Hill bolls which were set early in August had a period 10 to 12 days shorter than bolls set in September.

THE BEHAVIOR OF THE ANTHOCYAN PIGMENTS IN CANNING

By CHARLES W. CULPEPPER, *Associate Physiologist*, and JOSEPH S. CALDWELL, *Physiologist, Office of Horticulture, Bureau of Plant Industry, United States Department of Agriculture*[1]

INTRODUCTION

Many fresh fruits owe a considerable part of their attractiveness and consequent popularity to the bright red and purple colors of the anthocyan pigments present in the skin or flesh. It is desirable that the canned products made from these fruits retain the same brightness and freshness of color seen in the raw material. In the case of strawberries, raspberries, and cherries the degree to which the canned product retains the brilliance of color of the fresh fruit largely determines the desirability of a variety for canning purposes.

Much difficulty is encountered in securing satisfactory preservation of the natural colors in these and in many other fruits when canned, especially when the canning is done in tin. As a means of preserving more of the original color, the canning industry has resorted to the use of cans specially prepared by coating the inside with a lacquer designed to prevent contact of the contents with the metal. While the lacquered can permits much better preservation of color, it has proved a serious disappointment in another respect, as rapid corrosion of the metal occurs at breaks in the coating, and perforation of the can takes place much earlier than when plain tin is used. In consequence, the canner is forced to choose between placing heavily pigmented materials in plain tin, thereby losing in appearance but gaining in length of time over which the product may be held before consumption, or in lacquered cans, thereby gaining in appearance of the product but decreasing the time which will elapse before loss from perforation occurs.

A rather careful search of the literature has failed to reveal any detailed studies of the behavior of the anthocyan pigments in the course of canning processes. Various authors have mentioned the fact that fading or partial destruction of these pigments occurs, but they have advanced no suggestions as to the causes for the changes. The fact that pink, violet, or purple discolorations sometimes occur in peaches, pears, and other fruits has also been noted, but no investigations of the causes of such discolorations appear to have been made. Various tentative explanations have been offered, among which overprocessing is most frequently mentioned. Among other causes, the use of sunburned fruit (*8*),[2] of fruit grown in very hot localities (*10*), permitting fruit to become overheated during shipment and before processing (*8*, *10*), variety and degree of ripeness (*6*), and failure to cool promptly after processing (*16*), have been suggested. It has therefore been quite generally recognized that violet or pink discolorations are of occasional and sporadic occurrence in various

[1] Received for publication July 3, 1926; issued August, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 130.

canned fruits, but the reasons for such discolorations are very imperfectly understood, and it does not appear to have been suggested that the anthocyan pigments may stand in causal relationship to many cases of discoloration of this type.

The very extensive investigations of the anthocyan pigments made by Willstätter and his students (*34*, *36*, *37*) have shown that a single pigment may exhibit colors ranging from red to violet according as it is present in the free condition or as a salt. These workers have presented considerable evidence to show that many of the anthocyans form complex salts with metals, resulting in alteration in color. Shibata, Shibata, and Kasiwagi (*32*) have shown that in a very large number of anthocyans extracted from the colored parts of plants the addition of a metal to the aqueous solution produces a shifting of the color toward the violet end of the spectrum, the degree of alteration in color depending upon the metal employed. With aluminum sulphate and stannous chloride the colors produced, regardless of the original color of the pigment solution, are described as violet-red, pink, indigo, or lilac. That is, the action of either of these metals resulted in the production of some degree of violet color as a consequence of partial or total conversion of the free pigment into the violet-colored metallic salt. Nikiforowsky (*27*) considers that the color reaction with aluminum chloride is a specific reaction for anthocyans which permits them to be distinguished from flavonols and tannins. It is therefore well established that anthocyans generally, regardless of the color of the free pigment, react with many metals with an accompanying alteration of color toward the violet end of the spectrum.

It will be shown in the present communication that the presence in the material of anthocyans which react with the metal of the can is responsible for the discolorations characteristic of many fruits when canned in tin, and that the reaction of the pigment with the metal of the can is also largely responsible for the corrosion and perforation so characteristic of many of the pigmented fruits.

ANTHOCYAN PIGMENTS IN RELATION TO PINK OR PURPLE DISCOLORATION

In a number of fruits the anthocyan color is localized chiefly in the skin, and as a consequence of peeling is not usually present in the canned material. This is the case with the peach, the canned fruit usually showing no red or purple color. Any pink or violet discoloration which develops in the material subsequent to canning is therefore considered abnormal and often results in the discarding of the fruit as unwholesome and possibly poisonous.

A rather general appearance of discoloration in peaches put up by home canning clubs occurred in 1924 over a limited area lying between Athens and Augusta, Ga. Nearly all peaches canned in tin developed some degree of color, ranging from light pink to deep purple. In consequence, canning in tin was suspended, the trouble being popularly attributed to some defect in the tin cans being used.

The situation was brought to the attention of the writers by L. V. Strasburger, a chemist representing one of the can-manufacturing companies, who had spent several weeks in the territory in an attempt to find means of eliminating the difficulty. Strasburger

submitted a number of cans of material for examination, and described his experimental work in detail. He had employed a great variety of modifications in time and temperature of exhausting and of processing, in concentration of sirup used, and in degree of ripeness of fruit employed, but more or less purple discoloration was invariably present in his experimental packs. Substitution of hand peeling for lye peeling gave no better results. Tin cans from several different makers were employed, some of which had been left from a supply used with satisfactory results in previous years. No differences were noted, however, in the material in the different cans, color appearing in all in about the same degree. In glass, the fruit was in every case a perfectly normal, clear yellow color. As this suggested that the discoloration was produced by tin, Strasburger processed fruit in glass jars to which metallic tin or stannous chloride had been added. Discoloration identical with that occurring in cans resulted. At this point, Strasburger presented the case to the writers, stating his conclusion that the discoloration was due to a reaction of some constitutent of the fruit with the metal of the can, and asking their assistance in determining the exact nature of the trouble. A similar request was subsequently made by a number of canning club leaders and home demonstration agents. In consequence, the writers undertook to determine the class of compounds in the peach responsible for the trouble, subsequently expanding the work to include a considerable number of other fruits.

EXAMINATION OF PEACHES IN 1924

Material of the variety Belle of Georgia, canned by Strasburger at Athens, was subjected to careful examination upon its arrival at the laboratory. The riper pieces showed considerable bluish purple discoloration at the surface, and adhering bits of peel at the spray-burned tips were bluish in color. The firmer hard-green fruits were usually normal in color but occasionally were faintly violet. Purplish discoloration in some degree was evident in the stone cavity of almost every piece, but was much more pronounced in the riper fruits. The interior surfaces of the cans showed some corrosion but this was not excessive, being less than that found in standard commercial canned peaches purchased and opened for purposes of comparison. As it was noted that the discoloration apparently deepened in tint after the cans were opened, the fruit was emptied into shallow porcelain dishes and allowed to stand at room temperature overnight. The color was greatly intensified, the riper pieces becoming uniformly purple, while the liquid became so deeply purple as to appear almost black.

This condition recalled an observation made earlier in the season. In the course of a visit to some of the canning plants in the vicinity of Fort Valley, Ga., the writers had examined samples of unpeeled pie peaches showing a somewhat similar discoloration. The fruit, which was somewhat underripe, was merely washed with warm water after stoning, packed in the cans, and given the usual processing treatment. When some of these cans were opened it was observed that the bright red color of the skins had faded to a dull purple or muddy violet, and that the liquid in the can was a cloudy purple. This was recognized as an alteration of the skin pigments, and led

the authors to suspect that the discoloration observed in the peeled fruit might be due to the same cause.

Analyses made in the Bureau of Chemistry showed that the tin content of the discolored fruit, while somewhat in excess of that usually present in peaches examined so soon after packing, was not greater than that usually found in commercially canned peaches six to nine months after canning. Consumption of large amounts of the material by the writers showed that it was without harmful effects.

Some experiments designed to test the hypothesis that the development of purple discoloration was due to alteration in the anthocyans of the fruit were made on August 29, 1924, employing Elberta peaches grown at Arlington Experiment Farm, Rosslyn, Va.

The fruit was separated into two lots, one of which was soft ripe, the other in prime canning condition. The fruit was peeled by hand, packed in No. 2 plain tin cans, given a light exhaust, and processed 15 minutes at 100° C. A part of the material was packed with distilled water, a part with 30 per cent sirup, and a part with 30 per cent sirup to which 1 per cent of sodium chloride had been added. All the material was normal in color when opened except for a very slight purpling in the stone cavity in the case of the riper fruits packed in water or in sirup to which salt had been added. No change in color occurred when the material was allowed to stand in air overnight. The peels and stones from the fruit were packed in cans separately, water added, and processed exactly like the fruit. On opening, the peels showed different degrees of purpling corresponding to the degree of ripeness. The most intense color developed in peels from the ripest fruit, and the color became very intense on standing in air. The stones were intensely purple when the cans were opened. Additions of sodium hydroxide and ammonia in different amounts prior to canning decreased the discoloration of peels somewhat but did not wholly prevent it, and the material became deeply discolored on prolonged standing in air. Control lots of peeled fruits, peels, and stones were processed in glass. The fruit was normal yellow in color, the stones and liquid deep cherry red, while the color of the peels largely disappeared, the liquid becoming light red.

A quantity of stones were cracked, the kernels removed, and kernels and broken bits of stone were added separately to cans of peeled peaches prior to processing. The fruit with kernels added was normal except for a slight cloudiness of the liquid; that with broken stones had a deep purple liquor and the pieces of fruit were dyed superficially with the color.

These experiments pointed definitely to the red pigment as responsible for the discoloration, but indicated that contact with tin and subsequent exposure to air was necessary for its maximum development. They indicated also that the degree of discoloration depended directly upon the pigment content of the material. In consequence, some seedling fruit having a very intense red color extending from the skin into the flesh was employed for further work. Flesh, peels, and stones processed in tin showed considerably greater purpling at opening than did the Elbertas, and on standing overnight in air the flesh became a royal purple.

An aqueous extract of the pigment of the stones was made by heating a quantity of stones in water at 90° to 100° C. for 15 minutes. This yielded a deep wine-red solution. Heating with granular tin or adding dilute stannous chloride to this solution resulted in change of the color to very dark purple. Addition of citric acid to give a final concentration of 1 per cent preserved the red with very little change, and smaller amounts retarded the change in color to a degree proportional to the concentration.

From these experiments it seemed clear that the anthocyans of the skin and stone were responsible for the discoloration, and, as the pigment of the peach has never been investigated, it appeared advisable to make at least a partial purification of a quantity of it for a further study of its properties.

SEPARATION OF THE PIGMENT

About 3 kilograms of broken peach pits from which the kernels had been removed were extracted five times with glacial acetic acid. The extract was concentrated to small volume at 40° C. under greatly reduced pressure. The pigment was precipitated as a heavy sirup by the addition of about 10 volumes of ether, and the precipitate was washed several times with ether. It was then saturated with NaCl and extracted with butyl alcohol, repeated extraction being necessary to remove the bulk of the pigment. The butyl alcohol was washed several times with saturated NaCl, followed by three washings with a small amount of distilled water. The butyl alcohol was then concentrated under reduced pressure at 40° and the alcohol allowed to evaporate further at room temperature. The larger part of the pigment was precipitated from the butyl alcohol by the addition of several volumes of ether, collected on a filter, washed repeatedly with ether, and dried over calcium oxide. No attempt was made to crystallize or further purify the bright red powder. Its solution in water was almost neutral and was deep red with a tinge of purple. When the solution was rendered faintly acid, the color became deep bright red. Ferric chloride in nearly neutral solution gave a bluish black color in dilute solutions and a bluish black precipitate in greater concentrations. When the solution was made slightly acid, ferric chloride gave a purplish color, deepening to black. The absence of tannins, which would give a green color in acid solution with ferric chloride, was therefore indicated.

When the nearly neutral solution was heated with granular tin metal, slight darkening occurred, but there was no appreciable alteration of the red color. An acidified solution heated with metallic tin gave a deep purple color. With increase in the acidity of the solution the purpling decreased, practically no change occurring in strongly acid solution. When stannous chloride was added to a neutral solution, a blue precipitate was obtained. If the solution was slightly acidified and stannous chloride added, it developed a purple color, and a purple precipitate was thrown down. With increasing degrees of acidity the purpling decreased, strongly acid solutions remaining red with a faint tinge of purple. Aluminum chloride threw down a greenish blue precipitate from neutral solution; in slightly acid solution no change in color occurred. Lead acetate gave a greenish blue coloration in neutral solutions, but was without apparent effect upon

a solution acidified with acetic acid. Zinc chloride gave a green color in neutral solution, which returned immediately to red when the solution was made slightly acid.

From the results of this examination of the pigment it is apparent that the purple discoloration observed in peaches canned in tin is due to a reaction between the tin of the can and the anthocyan pigment present. It is possible that iron may be a contributing factor, as the pigment shows somewhat similar color reactions with salts of iron. The results also suggest that the acid relations are quite important, since the degree of acidity greatly modifies the intensity of the color.

The explanation of the fact that discoloration of the canned product is occasional and sporadic in its appearance, lies in the fact that the amount of the pigment developed in the fruit is determined by climatic conditions, hence varies considerably from year to year. In the district about Athens and Augusta, Ga., in which it was observed in 1924, the summer had been exceptionally hot and dry, with more than normal sunshine, and all varieties of peaches were unusually highly colored. In the Fort Valley district of Georgia the season of 1924 was close to normal in temperature and sunshine, and the commercial canners encountered little or no discoloration. The season of 1925 in the Fort Valley district was one of considerable excess of sunshine and rather high temperatures, and the fruit of all varieties showed exceptionally brilliant color. As a consequence, considerable violet or purple discoloration was observed in the commercial pack of several canneries in that district. It is clear that seasonal conditions determine the occurrence of the trouble by controlling the amount of anthocyan pigment developed in the fruit.

EXPERIMENTS WITH OTHER FRUITS

The work with the peach was considered as definitely establishing the fact that purple or violet discoloration of the canned product is due to a reaction of the anthocyan pigment of the fruit with the tin of the container. As somewhat similar discolorations had been observed by the writers in grape juices canned in tin, as well as in certain fruits, they were led to believe that the phenomenon might be of somewhat general occurrence in the more heavily pigmented fruits. In consequence, experiments were carried out with a considerable number of such fruits, including grapes, plums, red and black raspberries, blackberries, strawberries, cherries, mulberries, apples, fruits of Crataegus and Viburnum, Indian strawberries, and with beets and red cabbage. The results obtained with these materials will be briefly described. In all cases such an amount of material was prepared as permitted examination a few days after canning and again after six to nine months' storage.

Grapes

Uhland, a greenish yellow variety, was canned both in glass and tin. The two lots were indistinguisbable in appearance when opened, both being a dull grayish green. No change occurred on standing in air. Catawba and Rogers No. 13, both of which are red varieties, placed in plain tin cans and processed without exhaust-

ing, were, respectively, deep reddish purple and faintly purple when opened. On standing 48 hours in porcelain dishes the color of the liquid from tin containers became intensely purple and the flesh of the berries was dyed by it. In glass jars the color was a faint clear red which did not alter on exposure to the air. Four black grapes—Cloeta, Eumelan, Worden, and an unidentified variety—differed only in the intensity of the deep purple color developed in tin. In glass all were purplish red or brownish red, but when the liquid was removed and diluted with water it was seen to be a clear red with no suggestion of purple. Berries and skins of the deeply pigmented varieties were separated and processed separately in glass and tin. The berries in glass and tin were the same grayish green. Skins processed in glass were a deep wine red and neither skins nor liquid changed color on exposure to air. A portion of the wine-red liquid, when heated after the addition of a strip of tin foil, became light purple and on cooling deepened to intense purple. Skins in tin cans were a magnificient royal purple when opened, and the liquid drained from them, when added to the normally greenish gray pulps, stained them purple. When a few skins of one of the black varieties were added to a can of Uhland, the greenish yellow variety, before processing the fruit became a brownish purple.

After standing in storage 8 to 10 months, plain tin cans containing red or black grapes were intensely corroded. In the grapes canned without separating pulp and skins, the skins had faded to a dull buff color, but the flesh of the berries was dyed purple from the surface inward and broken berries were colored purple throughout. The liquid was distinctly purple and quite milky in appearance, apparently from the presence of hydrous stannous oxide. On exposure to air the milkiness increased and the purpling of both fruit and liquid became very intense.

The behavior of the grapes canned in tin is in many points identical with the disorder of wines known as blue casse. This condition is frequently encountered, especially by the French wine makers, in red or white wines which have been brought in contact with iron at some stage of the process of manufacture, usually in pressing. The trouble usually becomes apparent only after the wine is aerated, as in racking. In the case of a deep red wine, such as that made from Lenoir or other heavily pigmented grapes, the color gradually changes after racking through bluish red to dull violet, with a thin iridescent film at the surface. Ultimately a blue-black precipitate settles out, leaving the wine more or less altered in color. In white wines, the color changes pass through grayish or leaden shades to dull brown or brownish black. There is an enormous literature upon blue casse which will not be reviewed here. The essential facts thus far developed are that blue casse is the result of the presence of iron, either in the fruit or derived from the equipment employed in pressing and handling the juice, and that the iron combines with some constituent of the wine, subsequently producing abnormal color and going more or less completely out of solution upon exposure to air. It is significant that red and white wines, which may be made from the same variety of grapes, show differences in the colors developed. In the case of red wines, the fruit is crushed and fermentation is allowed to begin before pressing in order that a considerable part of

the pigment of the skins may be brought into solution. In white wines, heavily pigmented varieties may be employed, but the grapes are pressed cold immediately after crushing, in order that the pigments may not be carried into the wine. The differences in color of the red and white wines which develop casse may be due to the fact that in the red wines iron reacts chiefly with the pigment in solution, while in white wines the reaction is mainly with tannins.

A somewhat similar condition has been noted by Donauer (*11*) in the manufacture of Concord grape juice. He states:

> Concord grape juice is changed considerably in color by copper and copper alloys and nickel. The color is entirely destroyed by zinc or iron. Tin turns the color to a violet. Aluminum and its alloys or silver-plated metal are very satisfactory for such grape juice as far as its color is concerned.

The writers have found that the juices of any deeply pigmented grapes such as Lenoir, Clevener, Concord, Clinton, and Eumelan, whether fresh or pasteurized, give immediate color reactions upon the addition of ferric chloride, stannous chloride, or aluminum chloride in the cold, or by heating with metallic iron or tin. Upon the addition of stannous chloride the color is immediately transformed to purple or violet, according as smaller or larger amounts of the chloride are added. When acid is added the color returns toward red, finally becoming indistinguishable from the original juice. With aluminum chloride the change of color is less pronounced and the amount of acid required to produce return of the original color is much less. Ferric chloride produces an intense purplish black coloration, but the original color returns if sufficient acid is added. It may be pointed out that an identical treatment is employed with red wines which have developed blue casse, as they are acidified with citric acid and aerated, which restores much of the original color. There can be little question that reaction of the anthocyans with metals is responsible alike for the alterations in color and unfermented juices, in pigment-containing wines, and in the entire fruits when the materials are permitted to come in contact with metal.

Apples

Three varieties of apples were employed. Two of these, Hyslop and Small Red Siberian, are crab apples having intensely red coloration in the peel with white or light yellow flesh. The third, Baxter, in addition to having a deep red skin has the flesh more or less colored by red streaks extending inward almost to the core line. Small Red Siberian was canned whole in tin and in glass. In glass the red pigment dissolved in the water and gave a reddish solution, the fruits becoming clear yellow. In tin both fruit and liquid were faintly purple and became intensely purple on standing, the staining effect of the liquid upon the flesh being especially pronounced. The fruits of Hyslop were peeled by hand, the peels themselves being canned separately. The peeled fruit showed no change in the light yellow color during processing or after opening and exposing to air; peels and liquid become quite purple during processing and were deeply colored after overnight exposure to the air. The red striping in the flesh of Baxter faded almost completely in the material canned in glass; in the material in tin it went over to a rather faint purple which had diffused into the surrounding flesh. After 24 hours' exposure to the air, the color deepened to a decided purple in both liquid and flesh.

Crataegus Berries

Fruits of red haw processed 20 minutes in No. 2 cans had the color discharged, the fruits becoming bright yellow and the liquid very faintly red. On exposure to air the liquid became faintly reddish purple. In glass jars the fruit was yellow and the liquid clear, remaining unchanged after prolonged exposure to the air.

Viburnum Berries

Fruits of Viburnum sp. lost somewhat of their bright red color in processing and developed considerable purple on prolonged exposure to the air, but the development of the discoloration was much less rapid than in grapes, apples, or plums. Another sample of fruit which was greatly overmature and badly shrivelled, lost practically all color in processing and did not change to purple on standing.

Plums

Only one variety of plum, the Abundance, was used. The bright red-skinned fruits were canned whole. Both fruit and liquid were intensely purple when the cans were opened and the color deepened to purplish black, the change being more rapid than in any other of the various fruits tested.

Raspberries

Three varieties of raspberries were employed. These were the St. Regis, a variety having bright clear red fruits; the Cuthbert, a dark red; and an unnamed black variety closely resembling the wild black form. These were exhausted 2 minutes and processed 45 minutes at 85° C. In glass the St. Regis retained its bright red color. In tin it was very similar in appearance to the samples in glass when first opened but on exposure to air the liquid developed a reddish purple color, and the fruits above the liquid became a very dark purple.

The Cuthbert and the black variety were canned in both plain and enameled tin, with controls in glass. The Cuthbert in glass was a clear dark red. The fruit in enameled cans showed considerable fading of the original color and had a distinctly purplish tint when first opened. After exposure to the air it went over to a purplish red distinctly darker than that of the fruit in glass. The loss of color was much more pronounced in plain tin than in enameled cans, and there was more purple apparent on opening. On standing the color became dark purplish red like that of the enameled cans.

The black raspberry underwent very little change in color in glass. In enameled tins the color was only slightly less good than in glass, but had a faint purple tinge. In plain tin the original color was largely replaced by deep purplish red. On standing in air the fruit from both enameled and plain tins became so intensely purple as to appear almost black.

Oregon Evergreen Blackberry

The color in glass and in enameled tin was deep dark red, the latter having a purplish tinge which was not present in glass. This increased only very slightly after 24 hours in the air. In plain tin the original color was markedly faded and the liquid was purple and milky, probably from the presence of hydrous stannous oxide, as the metal was greatly etched. After standing overnight the liquid was purplish black, with milkiness still persisting. A similar condition was observed in strawberries, cherries, cranberries, raspberries, and mulberries.

Strawberries

The variety of strawberry used was the Klondike, the treatment employed being a 2-minute exhaust and a process of 30 minutes at 85° C. In glass and enameled tin the color faded to an unattractive brownish red, the fruit in cans having a purplish tinge not present in that in glass. In plain tin the color was a dirty brownish red and the liquid had a turbid, milky appearance, with slight purpling. Very little change in color occurred in fruit from either plain or enameled cans on standing overnight in shallow porcelain dishes.

In connection with another study in the laboratory, the writers had canned small experimental packs of about 220 selections and strains of strawberries representing most of the commercial varieties. In this work both plain and enameled cans were employed. Examination of this material eight months after its preparation shows a very wide range of behavior. The only generalization which appears to be justified is that the use of enameled cans in all cases contributes in some degree to preservation of the original color of the material. Varieties differ widely in their behavior. In some cases the original red color is replaced by varying shades of brown or brownish red, without suggestion of purpling either before or after exposure to the air. In other cases some degree of browning, varying from slight to extreme in the different varieties, was accompanied by discoloration which ranged from barely visible violet to intense purple, and which deepened more or less on contact with the air. In every case material in plain tin showed more loss of the original red, more browning, and greater purpling, in cases where purpling occurred, than in enameled tins.

Cherries

A number of varieties of cherry, so chosen as to include both sweet and sour and to give a considerable range in the amount of pigment in the fruit, were used. These included three varieties of sweet cherries (*Prunus avium*), Baumann May (Guigne de Mai), Napoleon (Royal Ann), and Meeker; three varieties of sour cherries (*P. cerasus*), Early Richmond, Montmorency, and St. Medard, and one hybrid between *P. avium* and *P. cerasus*, Nouvelle Royale. Each of these was preserved in both plain and enameled cans and in glass. The fruit was exhausted 2 minutes and processed 45 minutes at 85° C.

SWEET CHERRIES

Meeker is a light red fruit of very low acidity, Napoleon is a yellow-skinned variety having a red blush, low in acidity. Baumann May was decaying badly and consequently was picked while underripe; the fruit used was heavily blushed with red and was quite acid. The three varieties behaved precisely alike in that all trace of red was lost in processing in glass, the fruit becoming a clear bright yellow. The fruit in both enameled and plain tin was likewise a clear yellow when opened, but developed a pinkish purple tinge in the liquid after standing overnight. There was a moderate degree of corrosion in the plain tins after eight months' standing in storage in the laboratory.

SOUR CHERRIES

Of the fruits of this type, Early Richmond was somewhat underripe; hence was lighter in color and higher in acidity than the others. Montmorency was a deeper red and less acid, St. Medard was a uniform dark red from skin to stone and was only mildly acid. In all three the fruit processed in glass lost somewhat in brightness of color through diffusion of the pigments into the water, but the color was a clear red proportional in intensity to that of the fresh fruit and there was no pink or violet tint. In enameled cans after eight months' storage there was more intense color in all varieties than in glass, but there was considerable purpling in St. Medard. In plain tin, the fruit of all the varieties showed pronounced fading after 8 months. The liquid was a faint pinkish red with decided milkiness in Montmorency and Early Richmond and purplish red in St. Medard. After standing overnight in porcelain dishes both liquid and fruit were decidedly purple in all cases, intensely so in St. Medard. The contrast between the fruit in plain and that in enameled cans was very striking. Very slight purpling occurred in Montmorency and Early Richmond from enameled cans, although there was considerable in St. Medard. Two cans of Montmorency, one in plain and one in enameled tin, which were sealed and processed without previous exhausting, showed considerably more discoloration than the corresponding exhausted cans, while the unexhausted plain tin can had greater milkiness than the exhausted plain tin.

The hybrid cherry, Nouvelle Royale, was not fully ripe; hence was lighter in color and more acid than is typical of this variety. Fruit in glass faded to a faint pink, that in plain tin became almost colorless. Enameled cans preserved much more of the color than did glass. There was a faint purpling in plain tin after standing overnight and an almost imperceptible deepening of the color in the material from enameled cans.

MULBERRIES

Three strains of mulberries selected by the late Walter Van Fleet were employed. They differed very little in color, acidity, or physical character. Their behavior in canning was identical. The color in glass after eight months' storage was a deep, dark red, very little, if at all, different from the fresh fruit. In enameled tin the color was very well preserved but slight purpling was present. In plain

tin the color was a deep bluish purple in both fruit and liquid and the cans were heavily corroded. After overnight exposure to air there was very little change in the material from enameled cans, but that from plain tin had become intensely bluish purple. The effect of enameled cans in preserving the original color of the fruit was nowhere more strikingly evident than in the mulberry.

Indian Strawberry

The Indian strawberry (*Duchesnea indica*) is bright scarlet in color, the pigment being confined to the surface of the fruit. Color is entirely lost during processing, both in glass and tin, the fruit becoming a dingy reddish brown, which does not change on opening and aeration.

Purple Cabbage

In glass, the pigment of purple cabbage is largely extracted by the water, forming a beautiful, clear wine-red solution and leaving the tissue faintly red. In plain tin both cabbage and liquid were reddish purple, which became more intense on opening. After 17 months' storage material in glass had not changed materially, while that in tin had become light brown, about the color of ordinary cooked cabbage, and the entire inner surface of the can was deep purple to purplish black in color, due in part to sulphide blackening and in part to precipitation of the purple pigment.

Beets

Considerable fading of color, but no purpling, occurs in beets both in glass and tin. No suggestion of purpling is evident on heating fresh beet with stannous chloride, but if sufficient alkali to neutralize be added and the material heated with stannous chloride, a bluish-purple color appears and is followed by the throwing down of a purplish-black precipitate.

Cranberry

In cranberries canned in water, the liquid and the fruit became a clear deep red. Fruit in enameled cans differed very little from that in glass, but there was a slight purpling on occasional berries and a faint purple tinge in the liquid. After standing over night there was slight deepening of the purple tint in both berries and liquid. In plain tin there was very marked shifting of color from red to purple when the cans were opened four days after processing, and the color began to deepen immediately upon exposure to air, becoming pronouncedly purple overnight. The broken berries browned considerably as a result of oxidation of tannin when the fruit was emptied into a dish, and as a consequence the color of the fruit after exposure to air was blackish purple, the surrounding liquid being dark purple.

Summary of Results

The results of these experiments with various fruits show that the appearance of different shades of pink, violet, or purple during processing in tin cans, followed by intensification of the purple color upon exposure to air, occurs in a wide variety of fruits having red or

black-red anthocyan pigments present in skin or flesh. In no case does such discoloration occur in fruits known to be free of anthocyans, or in the colorless portions of anthocyan-containing fruits. In consequence, the appearance of some degree of alteration toward purple when the material is brought in contact with tin would appear to be a general and characteristic reaction of the anthocyan pigments, which will be encountered in canning or other manufacturing processes which involve contact of the material with tin.

When anthocyan-containing material which has been canned in plain tin is stored for six to nine months, the color upon opening is decidedly bleached as compared with like material opened a few days after canning. Material opened shortly after canning becomes strongly purple when exposed to the air for a few hours; that which has been canned for some months and which, consequently, has become bleached or faded, undergoes a return of the color toward that present immediately after processing, but this is accompanied by more intense purpling than occurs in material opened just after canning.

Kohman has stated that anthocyans are bleached with the tin of plain tin cans, but that the original color can be restored by exposure to the air (*18*). It must be said that the writers have found in this work that the color which develops on exposure to air is not the same as that originally present in the fresh fruit or that present in the material after processing in glass, but that there is a distinctly deeper color, due to the presence therein of more or less violet, converting the original clear red to purple. While in very acid fruits the alteration is less marked than in those of low acidity, it is usually sufficiently great to be observed when the container is opened, and can scarcely be overlooked after the material has remained a few hours in contact with the air.

CHEMISTRY OF THE ANTHOCYANS AS RELATED TO THEIR BEHAVIOR IN CANNING

In order to secure more information as to the mechanism of the discoloration, aqueous extracts of the various fruits employed in the experiments just described were prepared by boiling a quantity of the fruit with water, expressing and filtering the liquid, dividing into portions, and modifying the hydrogen-ion concentration by graduated additions of citric acid or sodium hydroxide. The degree of color change produced by treatment of the unaltered juice with stannous chloride, ferric chloride, and aluminum chloride could readily be compared with the effect of similar treatments upon the same extract after addition of acid or alkali.

The Grape

The pigments of the grape have received considerable attention. Willstätter and Zollinger (*39*, *41*) have examined varieties of *Vitis vinifera*, and Anderson (*1*, *2*) and Anderson and Nabenhauer (*3*, *4*) have studied varieties representing *V. labrusca*, hybrids of *labrusca* with *aestivalis* and *vulpina*, and a hybrid containing strains of *vinifera*, *aestivalis*, and *rupestris*. The results of these investigators show that the pigments present in these varieties, representing most of the species from which cultivated grapes have been derived, are

members of the delphinidin group, being either oenin (delphinidin dimethyl ether) or derivatives closely resembling oenin.

Of the seven varieties of grapes employed by the writers, the greenish yellow variety Uhland appears to contain no anthocyan pigment, as no violet or purple color developed when aqueous extracts were treated with stannous chloride, either with or without modification of the hydrogen-ion concentration. Extracts of the two red and four black varieties examined [3] differed only in the intensity of the color developed. Extracts of the red varieties became faintly purple, those of black varieties deeply purple. The color developed in partially neutralized extracts was in all cases much more intensely purple. In acidified extracts the change became less and less marked with increasing acidity. It is to be expected that members of the delphinidin group of the anthocyans will show the color reaction here described, wherever they may be found.

The Cherry

Keracyanin, the coloring matter of the sweet cherry, *Prunus avium*, has been studied in a preliminary way by Willstätter and Zollinger. Its properties in so far as known appear to identify it with the cyanidin group of anthocyans (*40*). It appears to be partially converted into colorless form by heat, whether in presence or absence of tin metal or salts.

Aqueous extracts of both sweet and sour varieties of cherries were tested with stannous chloride, aluminum chloride, and ferric chloride. The purpling with stannous chloride or aluminum chloride and the blackening with ferric chloride were very intense in the extracts of the deeply pigmented sweet varieties, slight to moderate in those of the sour varieties. That the color change in the sour varieties is inhibited by the acidity of the extract is shown by the intense purple developed after partial neutralization. Upon the gradual addition of acid, the purple color developed with tin or aluminum or the blackening produced by iron progressively lessened, and sufficient acid restored the original clear wine red. As in other cases, the alternate addition of acid and alkali, with the resulting shift of color, might be continued indefinitely.

The Raspberry

The pigment of the raspberry has been assigned by Willstätter and Bolton (*35*), on the basis of qualitative reactions, to the cyanidin group. Their only statement with respect to it is, "Auch die Himbeere und die Frucht der Eberesche (Vogelbeere) enthält ein Cyanidinglucosid," no report of the tests being made. In its behavior with tin the pigment resembles those of other cyanin-containing fruits. Aqueous extracts of fresh raspberries, as well as juices from fruits preserved for one year by processing in glass, were examined. Addition of aluminum chloride produced no change in color. With stannous chloride there was slight purpling. On the addition of an alkali, the juice to which no metal had been added became greenish

[3] Uhland is a hybrid of *Vitis labrusca* with *V. riparia*. The two red varieties, Rogers No. 13 and Catawba, are *labrusca-vinifera* hybrids. Of the black varieties, Worden is pure *labrusca*, Eumelan a *labrusca-vinifera-aestivalis* hybrid, Cloeta a *lincecumii-rupestris-labrusca-vinifera* hybrid, while the parentage of the unidentified variety, which was employed by mistake for a named variety, is unknown.

black; that to which aluminum chloride had been added became distinctly purple; that with stannous chloride very deeply purple. By adding the alkali drop by drop, the color in the presence of either metal undergoes a progressive change from red with a barely perceptible tinge of violet on the addition of the first drop to intense purple as the neutral point is reached. When acid is added the original color, whether in presence or absence of metal, returns. By alternately adding acid and alkali the shift of color may be produced at will. This behavior is characteristic of both cyanin and delphinidin pigments encountered in this work. It would appear from the writer's observations that in order to secure the characteristic color reactions with metals, the acidity of the solution must be somewhat less in the case of cyanins than is necessary with delphinidins.

The Cranberry

The cranberry contains a cyanin pigment, idaein, which is a monogalactoside of cyanin (*38*). The juice of freshly cooked cranberries was found by the writers to give no change in color when stannous chloride or aluminum chloride was added. On bringing the juice nearly to neutrality either of these reagents produces a faint purpling followed by the deposition of a purple precipitate. Iron chloride produces little or no change in the freshly cooked juice, but on partial neutralization a brownish black color develops after a few minutes standing in air.

The Beet

Betanin, the pigment of the red beet, has been isolated by Schudel (*31*), who found it to be unique among anthocyan pigments in that the glucoside contained nitrogen to the extent of 8.6 per cent. It consequently appears that it does not belong to any one of the three groups of anthocyan pigments thus far established. This is significant, since very little alteration in the quality of the color was observed in the canning tests. The pigment apparently undergoes partial destruction or conversion to colorless form on heating, as considerable decrease in intensity of color occurs both in glass and tin. It does not show any purplish discoloration with metallic tin or stannous chloride after heating and exposure to the air. If fresh beets are sliced and cooked in presence of granular tin, the color is identical with that of controls cooked in distilled water. If cooked with granular tin and a little acetic acid, there is pronounced fading of color, and purpling at the surface of the liquid accompanied by formation of an iridescent film occurs on 48 hours' standing. If stannous chloride is added to the cooked beets and the solution is then neutralized, purple color develops, and a purplish black precipitate is thrown down from a faintly purple solution. The behavior of the pigment of beet with tin differs markedly from that of the pigment of the sweet cherry and of the grape.

The anthocyans of the other species included in this study have not been sufficiently studied with respect to their chemical composition to make it certain whether all of them are derivatives of pelargonidin, cyanidin, and delphinidin, singly or in mixture, or whether some of them are derivatives of yet other fundamental com-

pounds. Detailed chemical investigation will be required to determine the nature of the pigment or pigments present in each case, since qualitative tests upon the crude pigments do not warrant the drawing of conclusions as to their chemical affinities, as Onslow (*28*) and Perkin and Everest (*29*) have pointed out.

The Strawberry

The strawberry material examined included more than 200 selections and strains representing a large number of varieties, and was of very widely differing character with respect to degree of acidity and amount of pigmentation. There was, consequently, no uniformity in behavior of the material canned in tin, as has already been stated. Material of Portia, a rather deeply colored berry, was employed in the tests of aqueous extracts with metals. It showed the same general behavior with respect to aluminum chloride, stannous chloride, and ferric chloride as the sour cherries. Addition of alkali intensified the purple color; addition of acid resulted in return to the original red. Material of Howard, a variety which contains a relatively small amount of pigment, was also employed. This berry faded in processing, even in glass, to dull brownish red. Juice of such material gave inconclusive results with stannous chloride and aluminum chloride, only a very faint purpling occurring with the former and none with the latter. The addition of acid caused the disappearance of the purple produced by tin salts, but the solution remained a dull brownish red with no intensification of the red. It is known from the work of Willstätter and his pupils that many anthocyans readily isomerize to colorless forms. It is also known that the presence of acids depresses or prevents the formation of the metallic compounds. In strawberries with a small amount of pigment and considerable acidity, isomerization, together with the browning of the tannins and the effects of the acid present, may be responsible for the difference in behavior from deeply pigmented forms, both in the can and in the presence of salts of tin and aluminum. The facts observed do not warrant the formation of an opinion as to the class of anthocyan pigments to which the color or colors of the strawberry belong.

Other Materials

In the other materials employed the color developed in tin was pink, pinkish red, purplish red, or purple, and in all cases intensification of the purple hue occurred when the material was left exposed to the air. The degree of alteration of color was in many cases clearly dependent upon the amount of pigment originally present in the fruit, faintly colored fruits giving faint pinks which deepened slowly to purple, as in the case of viburnum berries or light-red sour cherries. In Crataegus berries, the pigment is confined to the epidermal layers and is small in amount, and it is not clear whether the very slight purpling observed is due to partial destruction or decolorization of the pigment or merely to its small amount.

In the plum, raspberry, blackberry, mulberry, and red cabbage, the degree of alteration of color developed appears to be determined by the amount of pigment, but the resulting color is in every case some shade of purple. It ranges from rose red in the cranberry to a nearly pure violet in the mulberry, exposure to the air resulting in all cases in the intensification of the purple hue. With stannous chloride and aluminum chloride the same changes in color as described for

grapes, peaches, and cherries were observed, and when acid and alkali were added the behavior was similar to that in these fruits.

It seems clear that pigments belonging to both the delphinidin and cyaninidin groups show the same general behavior in tin, and their aqueous solutions show color reactions of the same general character when treated with tin and aluminum salts, namely, a shifting of color toward violet with alkali and a return to red with acid. Minor differences in behavior have been noted. It is possible that there are differences in the readiness with which the reaction takes place, depending upon whether a cyanidin or a delphinidin pigment is concerned. There may also be differences between members of the same group depending upon the state of the pigment, whether free or existing as mono- or di-glucoside. In so far as the pigments of the fruits here dealt with are known, nothing has been observed which can be considered as a specific reaction for any particular pigment, or for any one of the various forms of combination in which the pigment is known to exist. The pigment of the red beet is an exception, its behavior differing markedly from that of the other materials examined.

The behavior of the anthocyan pigments of fruits and vegetables when canned in tin is in complete agreement with the results of Shibata, Shibata, and Kasiwagi (*32*). The writers have found that the effect of heating anthocyan-containing materials in a container having a coating of metallic tin, or in glass in presence of metallic tin, tin salts, or aluminum salts, is to produce some degree of modification of the original color toward purple in the case of every material tested, with the exception of those cases in which the pigment is totally converted to colorless form under heat. If the effect of the combination of anthocyans with metals is to bring about a shift of color toward the violet end of the spectrum, regardless of the chemical constitution of the particular pigment concerned, the uniformity of behavior observed in a variety of fruits having pigments of unknown chemical structure is readily understood.

METHODS OF PREVENTING OR MINIMIZING THE ALTERATION OF ANTHOCYAN PIGMENTS IN CANNING

The difficulty encountered in securing satisfactory preservation of color in many materials when canned in plain tin has led to the introduction and extensive use of the inside enameled or lacquered can. George W. Cobb (*9*) states that this type of can, originally developed in Europe for the canning of certain meats and fish, was first employed for colored fruits in America in 1902 and that its use for red fruits and beets had become quite general by 1905. Bitting in 1912 (*7*) recommended its use for raspberries, cherries, plums, beets, pumpkin, and hominy. Such texts as those of Cruess (*10*), Zavalla (*42*), and Powell (*30*) recommend the use of the enameled can for acid or highly colored products. Merriman (*24*) has discussed the origin and chemical composition of various lacquering materials and has pointed out the limitations of their usefulness.

Concurrently with the introduction and adoption of the enameled can the canning industry has sought to continue the use of the less expensive plain tin can for some of the colored fruits by selecting for canning purposes the more deeply pigmented varieties or strains,

in the hope that a satisfactory degree of the original color might be retained when the material was preserved in plain tin. The results have been in part successful, in that a number of varieties of these fruits at present employed by canners can be put up in plain tin with a degree of preservation of color which makes them acceptable to consumers. In another respect the results have been disappointing. In the case of many fruits, the employment of the more heavily pigmented varieties of fruits results in more rapid generalized corrosion of the plain tin can or in more rapid pinholing of the enameled can than was encountered in the case of less deeply colored varieties of the same fruits. Attempts to increase the resistance of the can by modifications of the composition and methods of manufacture of the tin plate have been less fruitful than was hoped for (*6*, *12–15*, *25–26*). In consequence, the problem of corrosion and perforation is at present a more serious one than at any time in the history of the canning industry.

In the experiments here described, as well as in many others carried out in this laboratory in the past six or seven years, the anthocyan colors have in all cases been somewhat better preserved in enameled cans than in plain tin. In a few fruits, such as cranberries, the improvement due to the use of enameled containers, although distinct, is markedly less than in most materials. Even in the best enameled cans there is in all materials more loss of color than occurs in glass. Material canned in plain tin and opened a few days after processing shows considerable purpling and a milkiness of the liquid, neither of which is apparent in enameled cans of the same material opened after a like interval. When storage for six to nine months precedes the examination, considerable fading of the color is apparent in enameled cans, but is very much more pronounced in plain tin. On exposure to the air the material from plain tins undergoes marked intensification of color, finally approximating the purple tint observed on opening soon after processing. In enameled cans, some purpling occurs, but its amount is directly proportional to the area of metal exposed by imperfections in the lacquer.

ANTHOCYAN PIGMENTS IN RELATION TO CORROSION AND PERFORATION

The experience of canners with the various types of enameled cans and with plain tin as containers for the highly colored fruits and vegetables has led to recognition of the fact that the use of either involves difficulty. In the plain tin there is generalized corrosion of the inner surface with attendant loss of color on the part of the contents. In enameled cans there is better preservation of color but corrosion is localized at imperfections in the enamel, with the result that pinholing occurs in a relatively short time. In discussing this situation Baker (*5*) lists a number of fruits and vegetables which present especial difficulty. At the head of the list he places blackberries with strawberries next. The packing of these products he characterizes as gambling, not as a business. Rhubarb, blueberries, cranberries, black and red raspberries, loganberries, and blackberries are listed in the order of decreasing difficulty from the standpoint of perforation of cans. Baker recommends that those

which are sufficiently deeply colored to endure the resulting loss of color, such as blueberries, cranberries, and black raspberries, be placed in plain tin to prolong the period prior to perforation. Others which are not so heavily pigmented may be placed in enameled cans to preserve the color and considered as perishable goods by reason of the certainty that perforation will sooner or later occur. In making this recommendation of the use of plain tin for materials of this character Baker has left entirely out of account one very important consideration, namely, that the continued action of the material upon the surface of the plain tin may result in such an extensive absorption of tin by the contents that the wholesomeness of the material will become questionable.

In the experiments with the pigments of various fruits described in the preceding section, it became evident that the acidity of the solution was a very important factor in determining the amount of change in color produced by the addition of metal. In highly acid solutions there is little color change for the reason that the formation of the metallic salt of anthocyan is depressed or inhibited. It might be inferred from this observation that in highly colored fruits, those of higher acidity would exert less corrosive effect upon the metal of the can than those of low acidity. For the purpose of studying the action of some of the anthocyan-containing fruits upon the container in relation to such factors as acidity, tannin content, and oxygen content, experimental packs of all the fruits examined with respect to behavior of their pigments with metals, together with a number of the nonpigmented materials, were canned in both plain and enameled cans. A part of the material was exhausted prior to sealing, a part sealed without exhaust. The cans were then stored for a comparative study of the amount of corrosion. A part of the material was opened at the end of six months, a part at the end of nine months, and the remainder was held for observation as to perforation. The study of this material brings out a number of facts in regard to the factors affecting rate of corrosion in pigmented fruits.

A comparison of exhausted and unexhausted cans six to nine months after canning shows very clearly that oxygen content is an important factor in promoting corrosion. In every case, regardless of the pigment content or of the acid content of the material, the cans which had not been exhausted showed more extensive corrosion than did the exhausted controls. This is in agreement with the work of Kohman and others. The results indicate also that the presence of oxygen, as in unexhausted cans, may in certain instances lessen the production of free hydrogen. They indicate also that the presence of oxygen, together with anthocyan, greatly accelerates corrosion; that is, the effect of the two factors is additive.

The results with pigmented fruits show considerable evidence that corrosion of the can is materially decreased by the presence of high acidity in the material. The highly acid cranberry attacks the metal of the can much less extensively than do sweet cherries, blueberries, mulberries, strawberries, and black raspberries, all of which are much lower in acidity. The highly acid Oregon Evergreen blackberry does not attack the can as vigorously as does the black raspberry. Whether acid content is the sole factor responsible for the marked differences observed in these cases can not be stated defi-

nitely, but certainly it is a factor. Oregon Evergreen blackberries and Logan blackberries combine high acidity and heavy pigmentation, but do not attack the cans to the extent that would be expected if the corrosive effect of these two factors were additive. In the cases just cited, and in a number of others encountered in this work, high acidity to a very considerable extent nullifies the corrosive action of the pigment, despite the fact that high acidity is itself a factor which increases corrosion. Rhubarb, for example, produces intense corrosion of the cans in which its acidity is an important factor. In those materials which are highly pigmented and at the same time highly acid, the acid depresses the formation of metallic salts of the pigment and thus decreases the rate and extent of the solution of metal. A considerable number of facts long on record in the literature without accompanying explanation become intelligible in the light of the experiments here described.

Studies carried out by Leach (*23, p. 623–625*) long since brought out the interesting fact that canned blueberries, having an acidity approximating one-twentieth normal, dissolved and carried into solution several times as much tin in a given time as strawberries or raspberries having twice the titratable acidity. Leach also tested various concentrations of malic, citric, and tartaric acids for their ability to dissolve tin from tin plate, finding that the pure tenth-normal acids dissolved only about one-tenth as much tin as did blueberries of one-half the acidity in equivalent time. The action of the pure acid ceased after about three months, no more tin being dissolved after nine months additional exposure. No such stoppage of action occurred in blueberries, which were nine times as efficient in dissolving the metal as canned tomatoes and twenty times as efficient as the pure acids. Leach could give no explanation of these facts, which are readily understood if we conceive of the anthocyan as continuously removing metal from combination with the acid and thus preventing the attainment of equilibrium between free acid and tin salt which occurs in pure acid solutions. It also explains results such as those of Donauer (*11*), who determined the rate of solution of metal of kettles used for Pasteurizing grape juice, concentrating apple juice, and cooking tomato pulp. The temperature for the grape juice was that of Pasteurization, while the other products were boiled down to an acid concentration somewhat exceeding that of the grape juice. The amounts of tin dissolved in equal periods of service bore the ratios, apple juice 80, tomato pulp 115, grape juice 380. The rôle of the anthocyan as a metal acceptor is here clear.

The reason why fruits of very low acid content but with large amounts of pigment, such as black cherries and blueberries, bring about more extensive corrosion of plain tin or earlier perforation of enameled tin than do more acid, less deeply colored fruits, such as red raspberries or sour cherries, is also clear. In the presence of large amounts of anthocyan, salts of tin with the acids of the fruits can have only momentary existence since they are immediately decomposed with transfer of the tin to combination with the anthocyan. In consequence there is sustained attack by the acid which may strip the tin completely from the steel plate, yet the amount of acid salts of tin present in the material will be very low. In fruits of

high acidity and low anthocyan content the initial attack upon the metal is vigorous but the anthocyan is presently exhausted by combination with the tin, after which equilibrium between free acid and metallic salts is reached, and further action upon the metal ceases. The limiting factor in corrosion is not acid content, but anthocyan content, since the capacity for taking tin into stable organic combinations determines the extent to which it may be attacked.

Perforation

The material stored for the study of perforation was held at room temperature. The cans were in all cases stored on end, with the end which had been sealed after filling turned up. In all the experimental material, perforation was practically wholly confined to enameled cans. The percentage of hydrogen swells occurring in the enameled cans was also very much greater than in plain tin. In every case, enameled cans which ultimately showed perforation first became hydrogen swells. In only two cases did side perforations occur, in all others the perforation was located either in the countersink or at the juncture of the side seam with the end. In countersink perforations the opening is not at the bottom of the groove, but at the top, usually on the side next the center of the can. This is the region of maximum strain as a result of expansion during processing. The juncture of side seam and end seam is a region which is subjected to mechanical stress in the making of the cans, as well as to abrasion of the enamel covering. The location of perforations is therefore in accordance with expectation based on a knowledge of the behavior of metal previously subjected to strain when exposed to conditions favoring corrosion (*33*).

Packs of nonpigmented material prepared for comparison with pigmented material in the perforation studies gave no results since perforation failed to appear in such material.

It is apparent from the results of the author's comparative studies of the anthocyan-containing fruits that the amount of purple discoloration produced by contact with metal is a rather dependable indication of the ability of the material to perforate enameled cans. This holds true for the fruits of a considerable number of species which have been examined, and also for varieties and strains within a single species. Some 200 horticultural varieties and strains of strawberry have yielded evidence on this point. The most heavily pigmented varieties or strains were the first to perforate. Such deeply colored varieties as Portia and Progressive began to develop perforations after about six months, long before perforation began to occur in less highly pigmented varieties, such as Howard 17. The varieties of strawberries employed varied widely in acidity and in tannin content as well as in anthocyan content. The independent variations of acidity and tannin content complicate the results of the perforation study. Any substance which can take the dissolved metal out of combination with the acids of the fruit, thus setting the acid free to continue action upon the metal, will favor perforation or corrosion. Tannins and other substances capable of combining with the metal may function as aids to these processes just as anthocyans do. Consequently perforation, like corrosion, is favored by a variety of causes, of which the presence of anthocyan is only one.

The problem of discoloration is directly related only to such corrosion as is specifically due to the presence of anthocyans.

Kohman has given special attention to the problem of perforation by fruits containing anthocyans (*17–22*). He has shown that acidity of the fruit is not the most important factor determining perforation, since different products of like acidity but unlike pigment content show very large differences in the readiness with which they produce perforation. Kohman's results have led him to the belief that the rôle of anthocyan in perforation is that of a hydrogen acceptor—that is, the pigment takes up the hydrogen produced, so that perforation occurs without previous perceptible swelling of the can. The results obtained by the present writers indicate that anthocyan also functions as a metal acceptor, increasing the capacity of the material to dissolve metal by taking it into stable organic combination as rapidly as it is dissolved.

SUMMARY

Violet, pink, or purple discolorations in fruits and vegetables canned in tin are of rather widespread occurrence. In some materials such discolorations occur regularly, being accepted as a matter of course; in others their appearance is occasional and sporadic, resulting in considerable losses by causing the material to be discarded as unfit for food. Such discoloration has received various explanations, none of which are more than partially correct.

Discolorations of this type occur only in such fruits and vegetables as contain anthocyan pigments in skin or flesh, and are confined to the pigmented portions of the material. If pigmented and nonpigmented portions are separated prior to canning, discoloration appears in the pigmented portion but is absent from the nonpigmented portions.

The discoloration occurs only in material canned in tin, but occurs in both plain and enameled cans. When processing is carried out in glass, the original color of the material is preserved except for lessening in intensity due to partial conversion into colorless form by heat. In tin cans, a greater loss of color occurs, accompanied by conversion of some of the pigment to violet and consequent shifting of the color of the product toward purple. This alteration in color is intensified by opening the cans and exposing the material to the air.

The amount of anthocyan pigment developed in such fruits as have the pigmentation confined largely or wholly to the skin, as the red-skinned peaches, is determined in considerable degree by seasonal conditions. High temperatures and intense sunlight result in development of unusually high color in such fruits, and this results in the appearance of discoloration in the canned product.

The appearance of discoloration is due to the reaction of the anthocyan with tin dissolved from the container, and results in the formation of complex metallic compounds of anthocyan which are violet in color.

The anthocyan of the peach has been isolated and partially purified. Its behavior toward metals is similar to that of other anthocyan pigments.

The behavior of a large number of anthocyan-containing fruits and a few vegetables, including peaches, plums, sweet and sour cherries, apples, red and black raspberries, blackberries, currants,

grapes, mulberries, berries of Viburnum and Crataegus, red cabbage, and beets, has been studied by means of canning tests in plain and enameled tin and in glass; as well as the comparative behavior of aqueous solutions of the pigments with metals.

The formation of violet-colored salts with tin or tin salts is a general property of the red anthocyan pigments. It occurs when the anthocyan-containing material is heated in contact with tin, as in canning, when aqueous solutions of the crude or partially purified pigment are heated with metallic tin or aluminum, or when salts of these metals are added in the cold. The amount of the violet compound formed is determined by the amount of the pigment present, and by the degree of acidity of the medium, low acidity favoring its formation, high acidity depressing or suppressing it.

The color changes produced by combination with metals are reversible. Addition of alkali to a solution of pigment containing a metal results in formation of more salt with resulting intensification of violet color; addition of acid destroys the combination and restores the original red color.

In anthocyan-containing canned material the metal salts formed by combination of the acids of the fruit with the can metal are broken up by transfer of the metal into combination with the pigment, releasing the acid to continue attack upon the can. If the initial acidity is low, thereby favoring formation of the metal salt, extensive attack upon the can will occur, continuing up to the limit of the metal-absorbing capacity of the pigment. If the acidity is high, the formation of the metal-anthocyan compound is thereby reduced or suppressed, solution of the metal is limited to the absorbing capacity of the acids present, and equilibrium is reached before extensive corrosion occurs.

The enameled can preserves the original color of red fruits and reduces discoloration by decreasing the contact between pigment and metal. At the same time, it increases the rapidity with which perforations of the metal occur by limiting the area from which metal can be removed.

The processes resulting in discoloration are intimately related to the problem of corrosion and perforation, although these involve other factors. Anthocyan plays a very important rôle in causation of corrosion and perforation wherever it is present, but these processes can also be brought about by other compounds than anthocyans.

The principal factors concerned in corrosion are oxygen, acidity, anthocyan, and tannin. Some of these factors stand in antagonistic relationship. High acidity generally favors corrosion but depresses the formation of metal-anthocyan compounds and may thus retard corrosion. High acidity represses oxidation of tannin and formation of hydrous stannous oxide. Oxygen accelerates corrosion and increases the total activity by oxidizing the ferrous and stannous salts to the corresponding ferric and stannic states. The interrelationships of the factors concerned are so complex that practically every substance canned presents a specific problem.

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WHY APPLICATIONS OF NITROGEN TO LAND MAY CAUSE EITHER INCREASE OR DECREASE IN THE PROTEIN CONTENT OF WHEAT[1]

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INTRODUCTION

That low-protein wheat is due to the insufficiency of supply of nitrogen available to the plants during the later-growth stages has been shown by several investigators (*1, 2, 4*).[2] Thus it has been found that the application of soluble nitrogen to the plants at various stages of development up to a certain growth phase resulted in wheats that varied correspondingly in the protein content of the grain. The later nitrogen was applied, the higher was the protein content of the grain. However, as the literature also contains a fairly large number of references to investigations (*3, 5, 6, 7*) wherein the addition of nitrogen to land either failed to improve the protein content of wheat or actually caused a marked decrease in the percentage of this constituent, it is the purpose of this paper to set forth the conditions under which such results may be obtained.

Results obtained by the writer from investigations in which one of each of the essential salt elements was absent from the culture media of wheat for various periods of time following the initial exposure of the cultures to complete nutrient solutions, show that the absence of either of the two elements calcium and nitrogen, profoundly affect (decrease) the protein content of the grain. The percentage of protein in the grain obtained from cultures grown ten weeks in a complete nutrient solution and then transferred to media devoid of calcium was about one-half that obtained from cultures not deprived of any essential salt element; and in similar series in which nitrogen was omitted from the media, the grain contained about two-thirds the normal percentage of protein. Alkaline or neutral reactions of the media during the later growth phases of the plants caused a slight decrease in percentage of protein. The absence of potassium, phosphorus, or sulphur in the culture media after the plants had grown four weeks in complete culture solutions had no effect on the protein content of the grain. Similar experiments with magnesium showed that this element can be dispensed with after the plants have grown four weeks in complete culture media, with only slight decrease in protein. Thus it appears that variation in the protein of grain is primarily related to the nitrogen and calcium relations of the growth media.

FACTORS CAUSING VARIATION IN PROTEIN CONTENT OF GRAIN

The percentage of nitrogen or any other constituent in plant tissue is obviously a factorial measurement of the quantity of element that the plant has absorbed and of the growth it has made as the result

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[2] Reference is made by number (italic) to "Literature cited," p. 139.

and in part as an incidence of absorbing that quantity of the element. That the measure of growth (stated in terms of mass) plants make from any given quantity of nitrogen absorbed may vary markedly is only another way of stating that wheat varies in its percentage of this constituent. Thus, the problem of accounting for variation in the protein content of the grain resolves itself into a consideration of the factors which determine the mass of grain material that will be produced and into which a given quantity of nitrogen will be distributed and assimilated as protein.

Obviously nitrogen is required by wheat for the production of nongrain tissues as well as grain tissue. Furthermore, the quantity of grain tissue formed by a plant bears certain relations of proportionality to the quantity of nongrain tissue formed. The factors that bear on the apportionment of nitrogen to these two classes of tissue conceivably must be important in a consideration of the cause that effected the marked differences above referred to, in the responses from applications of nitrogen to land. In one set of cases fertilization with nitrogen gave rise to high protein grain and in another set to low protein grain.

DESCRIPTION AND RESULTS OF EXPERIMENTS

Marked differences were brought out very clearly by the writer in an investigation in which an array of varieties of spring wheats were treated with applications of sodium nitrate at various stages of their development. Furthermore, relations developed among the varieties that have made it possible to explain the paradoxical results of different investigators.

Briefly described, the experiments were as follows: Stone jars were filled with a fairly good soil and these were seeded with nine varieties of wheat. Each variety was treated separately, but all varieties were treated alike so that comparison could be made as to the effects of applications of nitrogen to different varieties at different stages of growth. The salt was supplied in solution upon the surface of the soil at a rate equal to an application of 100 pounds of nitrogen per acre. Soon after the seedlings appeared above the ground they were thinned to seven plants per jar. Mention is made of this at this time, as it will be shown later in this paper that the number of stalks produced by tillering had a very pronounced effect on the apportionment of nitrogen for grain and nongrain tissue. Table 1 summarizes the results of experiments made to compare the effect produced on the protein content of gain by nitrogen applied to different varieties of wheat at different stages of growth.

Several features of Table 1 need mention. It is to be noted that the percentage of protein in the grain of cultures which received nitrogen 30 or 90 days after planting was in every case greater than that obtained in grain from untreated cultures. With one exception, cultures that received nitrogen 90 days after planting produced grain higher in protein than did the cultures that received nitrogen at an earlier growth period. The exception referred to was noted in the case of Marquis.[3] The culture of this variety that received nitrogen 90 days after planting produced grain perceptibly lower in protein than did the cultures that received nitrogen 30 days after planting.

[3] This feature can be explained by a certain characteristic of the plant, namely, by the degree any treatment delays the maturity of the plants, the longer the delay the more remote is the application of nitrogen to the later growth period when it can fertilize nitrogen more efficiently for protein in the grain.

However, in either case the protein content was decidedly higher than that of the grain from untreated cultures. Disregarding this one exception, it may be stated that the markedly higher values obtained for the cultures that received nitrogen 90 days after planting, as compared with those for the cultures supplied with nitrogen at earlier growth periods is in general accord with the principle that the efficiency with which wheat plants utilize nitrogen for protein in grain is related to the proximity to the period of grain formation at which a given supply is absorbed by the plants.

TABLE 1.—*Percentage of protein in grain of cultures that received nitrogen at different growth stages*

Treatment	Varieties of wheat								
	Bunyip	Cedar	Dart's Imperial	Early Baart	Fulcaster	Hard Federation	Marquis	Sonora	White Australian
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
No nitrogen applied	8.94	9.51	8.28	8.76	8.34	12.21	8.50	7.88	9.0
Nitrogen applied at time of planting	9.60	10.67	7.35	8.84	7.32	9.79	8.92	7.15	9.0
Nitrogen applied 30 days after planting	13.62	12.14	10.76	10.98	9.33	12.05	11.60	9.81	9.2
Nitrogen applied 90 days after planting	15.54	15.55	11.38	13.03	11.90	14.76	11.00	11.98	12.1

The most interesting feature of Table 1, however, is the comparison it affords of the values for protein of the cultures that did not receive nitrogen and those that did receive it at the time of planting. This comparison shows that the values for protein of four varieties—Dart's Imperial, Fulcaster, Hard Federation, and Sonora—were less in the grain of the treated than in the grain of the untreated cultures. The values for White Australian in treated and untreated cultures were equal. Thus, in five of the nine varieties, application of nitrogen to the soil at the time of planting either had no effect or was more or less harmful to quality in wheat, as defined by the protein content of the grain. Table 1 shows that applications of nitrogen to plants at certain growth periods results in the production of high-protein grain, and likewise that applications of nitrogen at other times has a harmful effect. To explain these varying results it is necessary to consider the effect a given growth phase has upon the apportionment of nitrogen to the two classes of tissue, grain and nongrain.

Table 2 shows the relative increase in tillering of nine varieties of spring wheat resulting from application of nitrogen at different growth periods.

Table 2 gives important information concerning growth characteristics of different wheat varieties. It shows (what is well known) that varieties possess markedly different properties in respect to tillering. Differences were obtained in tillering within given varieties, as well as among varieties. The outstanding features, however, can be briefly stated as follows:

(1) All varieties except Dart's Imperial showed good tillering from nitrogen treatment.

(2) Dart's Imperial tillered very sparsely, regardless of the treatment employed. This wheat, because of genetic reasons, is considered incapable of tillering profusely.

(3) Bunyip, Cedar, Early Baart, Marquis, and White Australian, gave maximum tillering when nitrogen was applied 30 days to 90 days after planting. They tillered very sparsely when nitrogen was applied at the time of planting. Several characteristics of these varieties, however, need further mention, namely: They possess the ability to tiller, which is a heritable character of these varieties; their ability to tiller is conditioned on certain growth phases; tillering is dependent on an ample supply of available nitrogen.

(4) Fulcaster, Hard Federation, and Sonora tillered profusely with the first treatment, as well as with the later treatments. These varieties, therefore, differed from the ones mentioned above in that the growth phase did not condition the tillering properties of the plants.

It may be said, therefore, that tillering expresses both genetic and physiological properties of plants. As has been shown, however, variation in the protein content of grain of any given variety of wheat is due to physiological processes. If, therefore, tillering affects the protein content of the grain, it may be assumed to have some influence on the processes that affect the supply and apportionment of nitrogen to the grain and nongrain tissue.

TABLE 2.—*Relative increase in tillering of nine varieties of spring wheat due to application of nitrogen at different growth periods*

(Untreated cultures considered as basis, namely, 100)

	Bunyip	Cedar	Dart's Imperial	Early Baart	Fulcaster	Hard Federation	Marquis	Sonora	White Australian
Period after planting at which nitrogen was applied (days):									
0	104	133	102	109	126	140	117	204	116
30	171	212	108	214	129	156	218	188	140
60	194	224	127	267	150	198	197	219	156
90	275	259	115	267	160	258	197	213	186
Coefficient of tillering capacity *	2.64	1.95	1.13	2.45	1.27	1.84	1.68	1.04	1.60

* Obtained by dividing the highest value by the lowest value for each variety.

Tillering is essentially a process of vegetative development. Vegetative development precedes grain formation. If the supply of available nitrogen in the soil is less than the amount the plants can absorb and utilize, therefore it follows that excessive development of vegetative growth with incident demand on the limited supply of nitrogen accentuates the dearth of nitrogen during the later period of growth. Some of the nitrogen required for vegetative production can not be utilized later for protein in the grain. A certain minimum amount becomes a part of the nongrain tissue. The more there is of this tissue, the less is the quantity of nitrogen available for grain. But the amount of nongrain tissue that may be formed by a plant is limited by the length of the growth period. A given quantity and state of vegetative development precedes grain formation. Varieties like Fulcaster, Hard Federation, and Sonora that begin to tiller at very early growth stages have a relatively longer period in which to produce nongrain tissue than have varieties that tiller relatively late. Such varieties consequently can use relatively larger quotas of nitrogen for straw and leaves. If the supply is limited, obviously this would leave less nitrogen in the soil to be absorbed later for grain than in the case of those varieties that tiller relatively late in their life cycle.

Table 2 further shows that certain differences in the tillering properties of varieties have a definite relation to the variations in the percentage of protein resulting from the application of nitrogen at different periods of plant growth. Under the caption "Coefficient of tillering capacity of the varieties" is given the relative increases in tillering resulting from the application of nitrogen at a propitious growth period over that which was least propitious. It is to be noted that the lowest values resulted for Dart's Imperial, Fulcaster, and Sonora. These are three of the four varieties the untreated cultures of which produced grain higher in protein than did cultures treated at the time of planting.

The coefficient of tillering capacity as here applied is the quotient obtained by dividing the maximum tillering by the minimum. Low values may result (1) from varieties that do not tiller or that do it very sparsely at any growth phase (2) and from varieties that tiller equally abundantly at all growth stages. Both of these classes were represented among varieties that gave low values.

In Table 3, the data on tillering are analyzed further. The tillering percentage obtained from the cultures that received nitrogen 30 days after planting is divided by that of the cultures that received nitrogen at the time of planting. The values obtained give the tillering capacity of the varieties as a property of their early growth phase only.

TABLE 3.—*Increase in tillering of varieties of wheat resulting from the application of nitrogen 30 days after planting as compared to that obtained from the application of nitrogen at the time of planting*

Variety	Tillering increase from nitrogen application 30 days after planting	Variety	Tillering increase from nitrogen application 30 days after planting
	Per cent		*Per cent*
Bunyip	164	Hard Federation	111
Cedar	159	Marquis	186
Dart's Imperial	104	Sonora	92
Early Baart	196	White Australian	121
Fulcaster	103		

The four lowest values are those for Dart's Imperial, Fulcaster, Hard Federation, and Sonora. These are the four varieties that produced higher percentage of protein in the grain from untreated than from treated cultures. It should be noted that White Australian stands next in order to the four varieties above mentioned in percentage of protein produced in the grain. The percentage of protein in the treated and untreated cultures of this variety was equal.

As already indicated, the low tillering capacity values of the varieties may arise from one of two causes—(1) inability of the variety to tiller or (2) ability to tiller profusely at all growth stages. The relation between the low values of increase in tillering capacity and the value of protein in the grain resulting from the application of nitrogen 30 days after planting, brings up the question as to how these factors are related. It will be recalled that Dart's Imperial is representative of the first class, that did not tiller because of genetic

reasons. However, the plants of this variety did have the ability to absorb and utilize nitrogen equally well at all growth stages. Tillers were not necessary in plants of this variety to provide an avenue of increased vegetative growth as the result of adding nitrogen to the soil. The parent stalk at any growth stage had the capacity to expand sufficiently without tillering to take care of the increase in vegetative production that resulted from increased absorption of nitrogen. This is attested by the thick, heavy stalks this variety produced. Thus, the ability of Dart's Imperial to absorb relatively large quotas of nitrogen when supplied at the time of planting resulted in two reactions that precluded the production of high protein grain. In the first place the vegetative growth gave inception to too much grain for the nitrogen subsequently available; and in the second place nitrogen became deficient in the later-growth stage because the supply at the beginning of the season was insufficient to withstand the draft occasioned by the degree of growth this variety could make.

The Sonora variety is a representative of the second class of wheat which possesses the property to tiller equally abundantly at any growth stage, provided sufficient nitrogen (and, obviously, the other necessary elements) are available. Sonora could not expand sufficiently in the parent stalks to provide for all the vegetative growth resulting from the absorption of nitrogen during the early growth stages. Its tillers, however, developed very early. They had a longer growing period than did tillers that arose later; consequently they made a relatively greater draft on the supply of nitrogen in the soil than did the plants that tillered later. Capacity to absorb nitrogen may be considered as related factorially to the length of the growing period and to the mass of vegetative tissue produced. The relatively large vegetative development gave inception to more grain than would have resulted from a lesser vegetative development. Since the supply of nitrogen was insufficient for this increased quantity of grain, this grain was as a result lower in protein content than would have been the case had the yield been lower.

Inasmuch as the reason why the varieties Sonora and Dart's Imperial produced a higher protein grain from the untreated cultures than those to which nitrogen was applied at the time of planting is the effect which the early growth period has upon the subsequent supply of nitrogen, it is evident that both the soil factor and the plant factor are involved. It is clear, therefore, that by manipulation of either of two factors—i. e., by supplying nitrogen to the soil during the later growth period of the plant or by curtailing excessive grain production—it is possible to produce high-protein grain even under any condition.

Between Dart's Imperial and Sonora, representative of two types of wheats having distinctive genetic characters as to tillering, stand Fulcaster, Hard Federation, and White Australian. The growth habits of Fulcaster in respect to the features here discussed are more nearly akin to those of Dart's Imperial than Sonora. In respect to its ability to set tillers from applications of nitrogen at the time of planting, Hard Federation is similar to Sonora. However, it differs markedly from Sonora in that it has the property to increase in tillering capacity with growth. White Australian is a variety on the border line in respect to the effect which applications of nitrogen at the early growth stage have upon the protein content of the grain.

The treated and untreated cultures produced grain having the same protein values. Inspection of the data shows that the growth habits of this variety resemble those of Bunyip, Cedar, Early Baart, and Marquis more than they do the habits of the varieties in which applications of nitrogen at the time of planting produced lower protein values than were obtained for the untreated cultures.

CONCLUSIONS

It may be stated, therefore, that the supply of nitrogen available for the quantity of grain that any state of vegetative development may induce determines the protein content of any sample of wheat. It has been shown in this paper that the amount of nitrogen available to the plants at different growth stages affects the protein content of the grain. It appears, therefore, that some of the contradictory results obtained and reported in this field covering the effect of fertilizer applications on the protein content of wheat, may be explained by the peculiarities of the varieties. It seems well to emphasize here, also, a point that has not received the consideration in fertilizer experimentations that its importance merits, namely, that the properties of wheat varieties can and do markedly affect the efficiency of any fertilizer treatment.

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METABOLISM OF NITROGEN COMPOUNDS IN DORMANT AND NONDORMANT POTATO TUBERS[1]

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INTRODUCTION

In a previous paper by the present writer[2] it was shown that nitrates stimulate growth in dormant potato tubers to a greater degree than in nondormant tubers. The investigation herein reported indicates that the stimulation of growth by nitrates may not be due to an internal deficiency of soluble nitrogen compounds in dormant tubers that can be offset by the absorption of nitrate, as was suggested in the earlier paper.

EXPERIMENTAL DATA

INFLUENCE OF STORAGE TEMPERATURES AND NUTRIENT MEDIA ON LENGTH OF DORMANT PERIOD

One half of a uniform lot of immature tubers were stored at 5° C., the other half at room temperature (approximately 20°), and at intervals of two weeks representative samples from each group were planted in pure quartz sand. These quartz-sand cultures were divided into three groups. Two of the groups were supplied with complete nutrient solutions. The nitrogen in one occurred in the form of nitrate and in the other as an ammonium salt. The first solution contained the following salts in equal molal concentrations (0.007), KNO_3, $MgHPO_4$, and $CaSO_4$; the second contained equal molal concentrations (0.005) of KH_2PO_4, $(NH_4)_2SO_4$, $MgHPO_4$, and $CaSO_4$. The third group was supplied with a nitrogen-free solution similar to the first in composition and concentration except that KNO_3 was replaced by KH_2PO_4. Confirming the results obtained in previous experiments by the writer, Table 1 shows that the presence of nitrate tends to abbreviate the dormant period, but that the use of ammonium salts does not affect it; for there is no significant difference in the time of sprouting between the ammonium and the nitrogen-free cultures.

METABOLISM OF NITROGEN COMPOUNDS DURING THE DORMANT PERIOD

At intervals of two weeks during the dormant period samples of tubers were rapidly frozen, pulverized, and the juice expressed under approximately constant pressure (12.5 kgm. per square centimeter). The pulp residue was then extracted with distilled water and dried. The total nitrogen of this pulp residue progressively decreased, a total decrease of approximately 30 per cent occurring during the 10-week storage period at room temperature and approximately 20 per cent at 5° C. When the tubers were stored at room temperature

[1] Received for publication Mar. 22, 1927; issued August, 1927.

[2] Newton, W. The rest period of Solanum tuberosum in relation to available nitrogen. Science (n. s.) 58: 207-208. 1923.

the amino nitrogen in the expressed juice, as determined by the standard Van Slyke method, increased slightly with each successive analysis. The total increase during the 10-week storage period was from 15.4 to 17.5 mgm. of nitrogen per 10 c. c. of juice. When the tubers were stored at 5° the amino acid decreased slightly during the first four weeks, but this decrease was followed by an increase. However, at the end of the 10-week period the amino-acid content of the juice was not markedly greater than at the beginning. There was a significant progressive increase of amide nitrogen[3] in the expressed juice from the tubers stored at both temperatures; an increase during the 10-week period of from 4.9 to 7.2 mgm. per 10 c. c. of juice when the tubers were stored at room temperature, and from 4.9 to 6.7 mgm. when stored at 5°. Although the actual concentration of amino and amide nitrogen was greater in the expressed juice when the tubers were stored at room temperature, Table 1 indicates that the tubers stored at 5° tend to sprout first.

TABLE 1.—*Time from planting to sprouting of potato tubers treated with complete nutrient solutions and with nitrogen-free solution*

Number of weeks in storage	Temperature at which stored (° C.)	Date of planting	Average number of days from planting to sprouting (12 cultures)		
			Complete nutrient-solution cultures		Nitrogen-free cultures
			Nitrate	Ammonium	
2	5	Apr. 20	38	63	61
2	20	do	42	63	61
4	5	May 4	38	46	47
4	20	do	40	50	48
6	5	May 18	27	40	39
6	20	do	33	45	42
8	5	June 1	13	30	30
8	20	do	19	35	36
10	5	June 15	7	21	20
10	20	do	15	24	25

METABOLISM OF NITROGEN COMPOUNDS AS INFLUENCED BY STIMULI

The fact has long been recognized that stimuli which increase the rate of respiration in potato tissue will abbreviate the dormant period. Following the methods of Appleman[4] and Müller-Thurgau[5] the writer found that respiration was stimulated by (1) removing the skins; (2) treating the tubers with hydrogen peroxide; (3) storing at 0° C.; and (4) incubating at 35°. The expressed juice from the tubers thus treated contained 10, 12, 13, and 2 per cent more amino acid than the juice from tubers of the original stock. Since an error of 2 per cent was involved in the analytical procedure the last value may not be significant. In every case there was an increase of amide nitrogen as a result of these treatments; an increase of 10, 14, 36, and 8 per cent, respectively.

[3] The NH_3 evolved under slightly alkaline conditions after hydrolyzing for two hours with 4 per cent HCl at 100° C.

[4] APPLEMAN, C. O. BIOCHEMICAL AND PHYSIOLOGICAL STUDY OF THE REST PERIOD IN THE TUBERS OF SOLANUM TUBEROSUM. Md. Agr. Expt. Sta. Bul. 183, p. 181–226, illus. 1914.

[5] MÜLLER, H., *Thurgau*, and SCHNEIDER-ORELLI, O. BEITRÄGE ZUR KENNTNIS DER LEBENSVORGÄNGE IN RUHENDEN PFLANZENTEILEN, I–II. Flora (n. F. 1) 101: 309–372, illus., 1910; (n. F. 4) 104: 387–446, illus., 1912.

PROTEOLYTIC ENZYME ACTIVITY IN POTATO JUICE

Proteolytic enzyme activity was more intense in the expressed juice of nondormant than in that of dormant tubers, as indicated by the accumulation of amino acids upon incubating the juice under toluol at 30° C. For example, at harvest, and at four and eight weeks later, the increase of amino acid was 0.1, 0.9, and 2.3 mgm., respectively, in 10 c. c. of juice during a 48-hour incubation period. Results of this type were obtained only when the incubation period was short, for the initial increase of amino-acid nitrogen in the juice was invariably followed by a decrease. In one sample of juice the amino-acid nitrogen increased from 12.6 to 14.6 mgm. per 10 c. c. over a 24-hour incubation period at 40°, but over a 100-hour period the same juice contained only 13.5 mgm. This phenomenon, an increase followed by a decrease, was typical of the results obtained with all the samples of juice studied. The data from one sample of nondormant tuber juice was used to construct curve 2, Figure 1, to illustrate this general phenomenon.

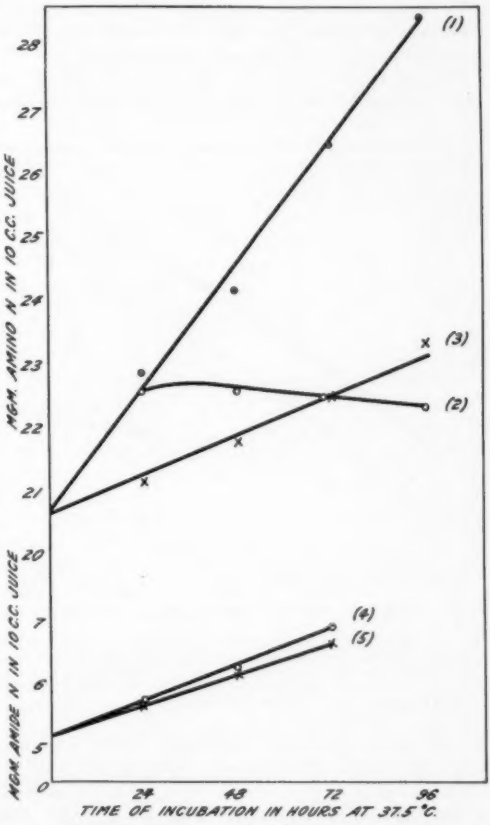

FIG. 1.—The influence of the addition of casein and calcium nitrate to nondormant potato juice incubated under 2 per cent toluol: Curve 1, juice containing 2 per cent casein; curve 2, juice alone; curve 3, juice containing 1 per cent $Ca(NO_3)_2$; curve 4, juice alone; curve 5, juice containing 1 per cent $Ca(NO_3)_2$

The disappearance of the amino-acid nitrogen may be accounted for by the enzymatic conversion of amino to amide nitrogen, for the amide nitrogen increased with the time of incubation and showed no maxima followed by a decrease. For example, during a 24 and a 100 hour incubation period of 40° C. the amide nitrogen in one sample of juice increased from 3.8 to 4.0 mgm. and from 3.8 to 4.3 mgm., respectively, in 10 c. c. of juice. Similar results were obtained with all the samples of juice studied. The data from one sample of nondormant tuber juice

was used to construct curve 4, Figure 1, to illustrate this general phenomenon.

Proteolytic enzyme activity may be demonstrated with greater ease by adding casein to the potato juice before incubating, for, unlike the results with the juice alone, after the addition of casein there was an accumulation of amino acids over a long period as approximately a straight-line function of the time of incubation. The data from one sample of nondormant tuber juice containing casein was used to construct curve 1, Figure 1, to illustrate the general nature of the results obtained.

When small quantities of calcium nitrate were added to the potato juice the rate of increase of both amino (fig. 1, curve 3) and amide nitrogen (fig. 1, curve 5) was inhibited. The inhibition was not so pronounced, however, in the case of amide nitrogen.

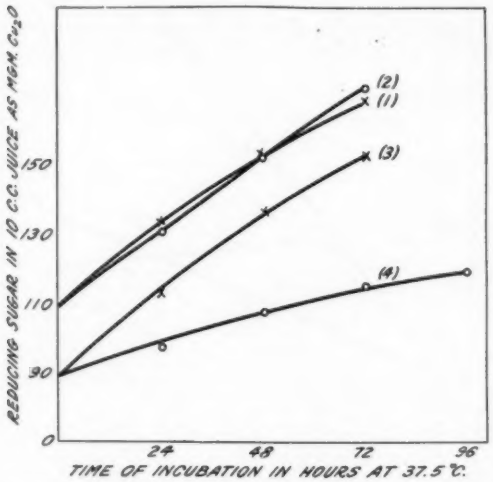

FIG. 2.—The influence of the addition of asparagine to nondormant and dormant potato juice incubated under 2 per cent toluol: Curve 1, nondormant juice containing 1 per cent asparagine; curve 2, nondormant juice alone; curve 3, dormant juice containing 1 per cent asparagine; curve 4, dormant juice alone

THE INFLUENCE OF ASPARAGINE ON ENZYME ACTIVITY

Probably the amide values recorded in the above experiments represent the amide group of asparagine or glutamine or both. Since the amide values were always higher in nondormant than in dormant tuber juice, there was a possibility that asparagine would have some influence upon enzyme activity in the expressed juice of dormant tubers. The addition of asparagine to dormant tuber juice increased the rate of accumulation of reducing sugar (compare curves 3 and 4, fig. 2), but had no significant effect upon nondormant tuber juice (fig. 2, curves 1 and 2). The reducing sugar was determined by the copper gravimetric method as modified by Davis and Daish.[6]

DISCUSSION

The stimulation of growth by nitrates may be due to the rule observed by Appleman,[7] namely, that oxidizing agents tend to stimulate growth in dormant tubers; for when the nitrogen of the nutrient solution was in the form of an ammonium salt no stimula-

[6] DAVIS, W. A., and DAISH, A. J. A STUDY OF THE METHODS OF ESTIMATION OF CARBOHYDRATES, ESPECIALLY IN PLANT-EXTRACTS. A NEW METHOD FOR THE ESTIMATION OF MALTOSE IN PRESENCE OF OTHER SUGARS. Jour. Agr. Sci. [England] 5: 437-468, illus. 1913.

[7] APPLEMAN, C. O. Op. cit.

tion occurred. Furthermore, no evidence was obtained to show that nitrate favored the accumulation of amino acid or amide nitrogen. On the contrary small concentrations of calcium nitrate strongly inhibited proteolytic enzyme activity and had a slight inhibitory effect upon the rate of the accumulation of amide nitrogen in expressed juice. Calcium nitrate was used because it proved to be less toxic than sodium or potassium nitrate when used as a single salt solution in cultural experiments. Although the specific effect of nitrates was not discovered by this study of the metabolism of the nitrogen compounds, evidence has been obtained that the cessation of the dormant period is dependent in part upon the activity of the proteolytic enzymes and the enzymes which convert amino into amide nitrogen.

Proteolytic enzyme activity may be measured by following in the juice the rate of accumulation of amino acid at low temperatures, provided the incubation period is short, but the results thus obtained are difficult to interpret because of the fact that the initial increase in amino-acid nitrogen is invariably followed by a decrease. The comparative proteolytic enzyme activity of samples of expressed potato juice may be followed with greater ease by adding casein to the juice. After the addition of casein the rate of the accumulation of amino-acid nitrogen was approximately a straight-line function of the time of incubation for periods as long as 96 hours at 37.5° C. The uniform rate of the accumulation of amino-acid nitrogen can not be explained by assuming that the original juice contained little protein, and hence that the addition of protein was required to demonstrate typical enzyme activity. On the contrary, large amounts of protein appeared to be present in the original juice, for upon removing the precipitate formed on heating the expressed juice, the total nitrogen was reduced to almost one-half.

Sure and Tottingham [8] have shown that amino acid may be converted into amide nitrogen. This phenomenon would serve to explain the decrease of amino acid which invariably followed the initial rise when expressed juice alone was incubated. The same phenomenon would also explain the apparent disappearance of amino acid during the first four weeks when tubers were stored at 5° C. Since the amide nitrogen content of the juice was invariably greater in nondormant than in dormant tubers there may be a possibility that amide nitrogen, possibly asparagine, is a factor upon which growth depends. The fact that the addition of asparagine to dormant tuber juice activates the carbohydrate hydrolytic enzymes but has no apparent influence upon nondormant tuber juice, tends to support this view.

SUMMARY

(1) The absorption of nitrates by potato tuber tissue abbreviated the dormant period, but under the same conditions ammonium salts did not affect it.

(2) There was a tendency for the amino and amide nitrogen to be greater in nondormant than in dormant tubers, but growth was not directly dependent upon the actual concentration of either of these compounds.

[8] SURE, B., and TOTTINGHAM, W. E. THE RELATION OF AMIDE NITROGEN TO THE NITROGEN METABOLISM OF THE PEA PLANT. Jour. Biol. Chem. 26: 535-548, illus. 1916.

(3) Proteolytic enzyme activity was more intense in the expressed juice of nondormant than in that of dormant tubers. When casein was added to the juice the rate of the accumulation of amino acid was a straight line function of the time of incubation.

(4) Evidence was obtained which indicated that when potato juice is incubated, amino acid nitrogen is converted into amide nitrogen.

(5) The addition of asparagine to dormant tuber juice activated the carbohydrate hydrolytic enzymes, but had no apparent influence upon nondormant tuber juice.

THE DETERMINATION OF QUALITY IN SWEET CORN SEED BY MEANS OF THE OPTICAL MEASUREMENT OF LEACHED MATERIALS[1]

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INTRODUCTION

In practice, injuries which impair the germination of seeds and the subsequent growth of the seedlings are ordinarily determined by means of actual yields from a field test, or, if this is not feasible, by the measurement of the heights, green weights, and dry weights of the seedlings as part of a greenhouse trial. The extent of such experiments and the time required for their completion imposes severe restrictions upon the number of samples which may be handled.

Several years ago, while working with sweet corn seed, the authors found it necessary to measure the injury caused by exposure to various temperatures. Limitations of time and space, as well as the large number of samples which required testing, made it imperative to devise a rapid and accurate method of determining viability and its relation to the subsequent vigor of growth in the seedling.

The senior author's observations made on seeds subjected to various temperatures and to chemicals in series of increasing concentration, showed that a marked correlation existed between the degree of injury to the seed and its susceptibility to fungous attack. The absence of fungous growth on the high quality control seeds and its progressive increase from the lower members in the series with only slight injury to the higher with increasing injury and final death, seemed to indicate that such growth is due to leachings from the seed. The measurement of such leached materials, inorganic and organic, crystalline and colloidal in nature, should serve as an index to the vigor of the protoplast and thus of the viability of the seeds. The effects of stimulation or injury on the permeability of the cell membranes have long been known. Osterhout (*11*)[2] and others in numerous experiments have recently measured the relation of conductivity and permeability to injury and death. They have found the electrical resistance of a plant to be an excellent indicator of what may be called its "normal condition of vitality." Any change in this condition is rapidly and positively shown by a change in electrical resistance. The method of Osterhout therefore received first attention. But before much progress had been made this was abandoned and others sought which would be more definitely applicable to the problem. It was essential that the methods used should measure not only the change in permeability of the membrane, but also the relative quantity of materials, crystalline organic and inorganic, and colloids, that are lost by diffusion through such changed membranes. In the conductivity method the permeability of the

[1] Received for publication Apr. 25, 1927; issued August, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 166.

membrane is determined by the behavior of the electrolytes, whereas for this problem it seemed more appropriate to determine the permeability by measuring the inorganic and organic crystalloids and colloids. The former were measured by the use of the refractometer and the latter by the use of the nephelometer. After two years of investigation the refractive index and the colloidal index tests have proven very serviceable. These have their individual merits, but in practice supplement each other to a certain degree. The studies have been confined to sweet corn, but others working in the same laboratories have found the tests to be equally applicable to maize, garden peas, and radish.

LABORATORY METHODS

In the methods about to be described, the authors believe that both time and temperature of soaking the seed prior to the readings play an important part, but lack of space prevents giving this phase of the problem the attention it deserves. In experiments of this nature it is important to determine the point where pronounced differences in the colloidal index are obtained. After a number of trials 48 to 72 hours of soaking at 30° C. seemed to give the best results, and either one or the other of these periods was used in most of the subsequent readings. The 48-hour period at 30° appears to give the greatest differences in colloidal index when the germination of the corn has been in some way impaired. In such cases the leaching action is much more rapid than in normal corn and the best readings can be made within 48 hours. Sweet corn which is apparently normal in all respects leaches more slowly, and the longer period of soaking, 72 hours at 30°, gives the more reliable readings. In practice the length of the period can be determined with great ease by the appearance of the liquids after 24 hours leaching. If the colloidal solutions are quite opalescent the corn need not be soaked longer than 48 hours. On the other hand, clear or only slightly opalescent solutions indicate that 72 hours' soaking is desirable. Dispersions from badly injured seeds appear very turbid, and if many of them are in this condition the readings may be taken at 36 hours or sooner.

In order to obtain uniformity of results and freedom from the salts present in tap water, the seeds were soaked in distilled water. This brings up the question of the effect of distilled water on the protoplast and on leaching. André (*1*) observed that the seeds of beans and wheat soaked in distilled water for periods up to 281 days lost considerable amounts of nitrogen, phosphoric acid, and potash. True (*13*), True and Bartlett (*14*), Merrill (*10*), Hibbard (*6*), and others observe that distilled water is toxic to the roots of seedlings. Hibbard (*6*) states that where disease-free seed uniform in size, color, etc., is selected from the pure line offspring of one plant, there is as much variation in the behavior of the seedlings toward toxic substances in solution as in a mixed progeny from unknown sources. Hibbard states further that the harmful action of distilled water to seedlings is due not to a single predominant factor, but to a combination of many. These factors he conceives as forces bringing about a disturbance of the normal equilibrium of the various chemical and physical interactions within the organism and between it and its environment. Merrill (*10*) gives three reasons which may account

for the toxicity of distilled water to plants: (1) The lack of essential nutrients; (2) the presence of deleterious substances; and (3) the extraction of salts, or nutrient materials, from the organism immersed in distilled water. True (*13*) has made extensive observations concerning the relation between growth and the leaching of electrolytes from the roots of lupine seedlings grown in distilled water. He shows that with roots the rate of leaching is greatest at the end of 24 hours and progressively decreases with each 24-hour interval. The growth rate in this culture falls off in a manner suggesting a parallel with the decrease in resistance.

André (*1*), with seeds of wheat and garden beans, shows a leaching effect which is similar in some respects to the loss of electrolytes from the seedlings of True (*13*). It is the work of André and True which suggested a possible means of measuring the vigor of seeds by determining the soluble solids which leach from them in distilled water.

The proportions of corn and distilled water used in experiments of this character require careful consideration. In corn of high quality and consequently low permeability a ratio of 5 c. c. of distilled water per gram of corn is sufficient. In other cases 10 c. c. of distilled water per gram of corn is advisable. In all of their tests the authors used either 5 or 10 gm. of corn in 50 c. c. of distilled water. Due to the necessity of using whole grains it is virtually impossible to weigh out rapidly and accurately less than 5 gm. of corn.

In making the nephelometer test a proper standard of comparison is absolutely necessary. Kleinmann (*7*) has pointed out that the standard solution should be capable of remaining constant for several months. This is true of many inorganic sols, but care must be exercised with reference to color.

The colloids leached from the Country Gentleman and Narrow Grain Evergreen varieties of sweet corn are either clear or opalescent. Very rarely, and in the case of badly discolored and diseased specimens only, the liquid is somewhat yellowish. The best results were obtained, therefore, by the use of a slightly opalescent standard. After testing a considerable number of standards, including those used by Kober (*8*), Richards and Wells (*12*), and others, the authors found that chemically pure soluble starch fulfilled all requirements except one, namely, its keeping quality. This was overcome by making up a solution containing 0.5 per cent soluble starch and 0.5 per cent sodium toluene para sulphochloramid. The keeping qualities of this standard are excellent. Since the soluble starch obtained from the manufacturer varies in quality, care should be exercised to make the standard for a given series of experiments from the same lot.

In soaking samples of sweet corn the presence of bits of silks and chaff, pieces of pericarp and other detritus is practically unavoidable. Blowing and cleaning the samples on a screen previous to soaking removes only the coarser particles. Previous washing in distilled water also fails to eliminate them. The presence of these foreign, noncolloidal materials, together with the large particles in suspension due to coalescence, seriously affects the accuracy of the readings. Folin and Denis (*5*) have shown that the presence of large particles due to coalescence causes gross errors in the nephelometer readings. A series of tests by the authors shows that these errors could be greatly reduced by filtering the colloidal solutions through a porous filter paper.

The authors maintained a uniform procedure in preparing the samples. The ears in each case were shelled into a small box without either butting, tipping, or removing any of the diseased kernels. The kernels were well mixed, cleaned by screening and blowing, and then weighed out. Wide-mouthed bottles of 150 c. c. capacity were used for soaking the seed. The weighed samples were placed in these bottles, the distilled water added, and the bottles stoppered to prevent evaporation of the liquid. Each sample was then thoroughly shaken in order to remove air bubbles from around the kernels and likewise to immerse those which floated. The corn was leached in large, accurately controlled temperature cases. The bottles were removed at the end of the required period and the liquid of each decanted into a funnel lined with a porous filter paper. The filtrate was allowed to run into 100 c. c. test tubes and the readings taken as soon as sufficient liquid had filtered. In most instances duplicate samples were weighed from each ear and two readings taken from the respective filtrates.

The character of the experiments is such that both refractometer and nephelometer readings were taken for many of the samples. The technic described thus far proved applicable for both tests. Filtering the liquid is of course not necessary if only a refractometer reading is taken, as the index is affected only by the solutes.

All readings were checked by means of growth tests on greenhouse benches. A duplicate random sample of 20 kernels from each ear was planted in a single row ½ inch deep and 1 inch apart, the rows being 2 inches apart. Each fifth row was planted to a control consisting of a random sample showing 100 per cent rag-doll germination, and testing relatively free from rots and molds. All the ears in a given test were planted the same day. Measurements were taken in a single day at the age of about 18 days. The germination was computed to a percentage basis by assuming that if each of the 20 kernels produced a seedling, the germination was 100 per cent. The failure of one kernel to grow reduced this to 95 per cent, of two kernels, to 90 per cent, etc. Each seedling was evaluated 5 per cent. The height of each plant was the measured distance from the ground to the tip of the longest leaf. Averages were calculated by dividing the sum of the heights by 20. After they had been measured, the plants in each row were cut off at ground level and weighed. This total weight divided by 20 represented the average green weight. The average heights and green weights have been compared in each case with the respective average heights and green weights of the controls. The resulting increases or decreases were then compared with their respective instrument readings, and the correlation coefficients determined.

METHODS OF CALCULATION

The many thousands of observations necessitate presenting results in extremely condensed form. For this purpose much of the subsequent data have been calculated as correlation coefficients according to the formula of Pearson:

$$r_{xy}=\left[\frac{(\Sigma d_x d_y)}{n}-w_x w_y\right]\left[\frac{1}{\sigma_x \sigma_y}\right]$$

in which r_{xy} is the coefficient between the characters x and y; d, the deviations; w, the correction factor; n, the number of observations;

and σ, the standard deviation. The probable errors of r_{xy} have been calculated from the formula

$$\text{P. E.}_r = \pm\frac{0.6745\ (1-r^2)}{\sqrt{n}}$$

In certain cases the standard deviations have been determined from the "short method" formula

$$\sigma = \sqrt{\frac{\Sigma(fD^2)}{n} - w^2}$$

in which f means the frequency, D means the deviation from the assumed mean, and w the correction factor.

The probable errors of the standards of deviation have been calculated from the formula:

$$E\sigma = \frac{\pm 0.6745\sigma}{\sqrt{2n}}$$

THE REFRACTIVE INDEX TEST

The Abbé refractometer provides a rapid and highly accurate means of determining the specific gravity of a liquid and the percentage of total solids in solution. Bigelow and Fitzgerald (*2*), basing their investigations on Windisch's tables, have shown that the specific gravity and total solids in tomato pulp bear the same relation to each other as in beer and wine extracts. Since such proportions have been found to exist in these and in other instances, it may be assumed that the leachings from sweet corn kernels are proportional in the same manner. With this assumption a series of experiments was undertaken using a Bausch & Lomb Abbé refractometer.

Tables 1 and 2 give the results of preliminary tests on random samples of seed treated in different ways for the purpose of showing the reliability of the test. The sweet corn used in this test was a random sample of Country Gentleman showing good germination and almost complete freedom from rots or molds.

TABLE 1.—*Comparison of the total solids in solution leached from sweet corn soaked in distilled water at various controlled temperatures for two different periods of time*

Temperature at which corn was leached	Refractive index (25° C.)	
	After 24 hours	After 96 hours
° *C.*		
30	1.3345	1.3354
30	1.3345	1.3352
20	1.3342	1.3345
20	1.3342	1.3345
10	1.3341	1.3343
10	1.3340	1.3342
Distilled water.	1.3325	1.3325

From Table 1 it is evident that leaching is progressive both as to time and temperature. The experiment could not be continued beyond 96 hours, because of the fermentation occurring in the 30° C. sample.

In Table 2 a comparison is made between samples of killed and viable Narrow Grain Evergreen sweet corn taken from a single lot of seed. This corn tested 95 per cent germination and was free from disease. Twelve 25-gm. samples were weighed out and six of these subjected to a temperature of 100° C. for one hour in order to destroy life. The readings in Table 2 are the averages of eight replications. Each lot was leached in 50 c. c. of distilled water at the temperatures designated. The results show clearly that the rate of leaching is much more rapid in the killed seed and, likewise, that the rate of loss in both killed and viable samples increases progressively with the temperature.

TABLE 2.—*Comparison of the loss of soluble total solids from viable and killed seed of Narrow Grain Evergreen sweet corn after 48 hours leaching*

Condition of seed	Temperature at which seed was leached	Average refractive index (21.5° C.)
	° C.	
Killed	30	1.33600
Viable	30	1.33515
Killed	20	1.33545
Viable	20	1.33470
Killed	10	1.33460
Viable	10	1.33455
Distilled water		1.332845

THE COLLOIDAL INDEX TEST

During the course of the earlier experiments it was noted that whereas in a few samples the liquid remained perfectly clear at the end of the soaking period, in the great majority it was more or less opalescent. Observations revealed that strong, vigorous growth was usually coincidental with a clear liquid, while the opposite was true of samples in which the liquid was opalescent. Test readings of such liquids were made with a Duboscq colorimeter converted into a nephelometer according to the method of Bloor (*3*). This instrument is capable of measuring with great precision the dispersity of a suspension such as is found when sweet corn kernels are treated with distilled water.

The corn was prepared as already described and the readings made with both tubes of the nephelometer nearly full and at exactly the same height, thus eliminating the necessity of volume corrections.

In order to determine whether colloids respond in a manner similar to the solids in solution, a number of experiments were undertaken, of which the results shown in Table 3 are representative. The samples for this test were prepared from the same lot and treated in the manner indicated in Table 1. At the close of the 66-hour period of soaking at 10°, 20°, and 30° C., respectively, the colloids increased

progressively with the temperature and had an extremely wide range. After 96 hours' soaking, however, the colloids as measured by the nephelometer had become nearly identical in quantity and the index in each case was very low. The respective suspensions gave visible evidence of the action of microorganisms.

It is quite likely that this is the principal reason for the lack of uniformity in some of the results given later. Subsequent work, not reported here, prevented such inconsistencies largely through the addition to the distilled water of a germicide that has no tendency to coalesce the colloids. In these investigations, however, the addition of a germicide has been avoided, as the primary object is to measure the normal leaching in sweet corn in no wise modified by the presence of a foreign substance, and its likely effect on changing the permeability of the membranes.

TABLE 3.—*Comparison of the loss of colloids from viable and killed seed of Country Gentleman sweet corn*

Temperature	Number of readings	Colloidal index (standard 30)	Time leached (hours)	Condition of seed
° *C.*				
30	4	12.40	66	Viable.
20	4	22.50	66	Do.
10	4	74.20	66	Do.
30	4	7.60	66	Killed.
20	4	13.00	66	Do.
10	4	43.20	66	Do.
30	4	2.32	96	Viable.
20	4	3.90	96	Do.
10	4	16.50	96	Do.
30	4	2.25	96	Killed.
20	4	2.40	96	Do.
10	4	5.85	96	Do.

Other methods of controlling the growth of microorganisms have suggested themselves, the most promising being the leaching for longer periods at temperatures which inhibit or prevent their growth, and yet are not injurious to the seeds. (Compare Tables 1, 2, and 3.)

Under conditions favorable to the rapid development of organisms, it may be surmised that the colloidal index becomes largely a measure of the dispersity of the microorganisms. That this is not the case is indicated by the samples in Table 3 which were leached at 10° C., a temperature not favorable for the rapid growth of bacteria and fungi. The relatively low colloidal indices of the sweet corn soaked at 10° for 96 hours can be interpreted as due solely to the presence in such samples of membranes impaired by disease or otherwise and the consequent free diffusion of cell colloids through them. This view is further supported by comparisons of the colloidal indices of killed and viable seeds in Table 3. Here the corn subjected to 100° for one hour was killed and a change occurred in the protoplast, including the organic membranes. At the same time, at least partial sterilization is indicated. Nevertheless, the colloidal indices of the viable, unsterilized samples are consistently higher. Table 3 represents only one of many similar tests, all of which have the same

trend. However, there can be no doubt that microorganisms do affect the suspensions. Whether the cause is physical (such as heat), chemical, or pathological, the weakened membrane permits the colloids and soluble solids to diffuse through it with greater freedom. Concomitantly, the growth of microorganisms, because of the greater abundance of food, has an upward tendency. This increased growth of microorganisms may actually raise the colloidal index by decreasing the colloids in suspension. This explains some of the discrepancies which will be noted later.

It is quite probable that improved technic and the use of more sensitive instruments such as the photometer, instead of the nephelometer, will further increase the reliability of this test. The authors have realized from the start that the use of filter paper in preparing the samples for nephelometer readings is open to criticism on the score that some of the suspension particles would be retained. The question is, as mentioned previously, whether filter paper is the source of greater errors for such uses as here outlined than those which are known to result from coalesced colloidal particles and the unavoidable presence of dust and refuse from the samples. In certain instances which, however, have not been observed more than a half-dozen times in thousands of readings made, the leachings are viscous and approach the nature of a gel. Such liquids are virtually unfilterable, and in cases where the index of the filtrate alone is taken it is invariably too high. Ears Nos. 51 and 55 in Table 10 are typical of this condition.

In this connection an error due to the instrument itself may be mentioned. Kober (*8*) and Kober and Egerer (*9*) show that the amounts of precipitate in solution are not exactly in inverse proportion to the calibration on the scale. In cases where the density of the precipitate in the standard solution and the unknown are within 10 per cent of each other (about 20 per cent when the depths of the liquids are 60 mm.) accurate readings can be taken directly from the scale. If greater differences occur, corrections are necessary. For this purpose Kober (*8*) proposes a formula which has been used successfully by other workers. Since the variations in the case of sweet corn samples are much wider than those encountered in the usual laboratory analytical work, it is obvious that very wide differences occur between the standard and unknown. In a relatively dense hydrosol, such as the standard used by the authors, the readings for the unknown solutions which contain a smaller percentage of colloids are subject to a greater error than in cases where the standard and unknown are within 10 to 20 per cent of each other. Kober (*8*) shows that when the ratios between standard and unknown are within 10 per cent of unity, the edestin curve and the hypothetical curve (which is inversely proportional to the concentration of the solutions) are within 1.5 per cent of each other. It is evident that if very accurate readings are required it is necessary either to adjust the volumes of the solutions as indicated by Richards and Wells (*12*), to use the formula of Kober (*8*) and Kober and Egerer (*9*), or to vary the concentration of the standard.

The application of Kober's formula in this instance is open to question because of the fact that the composition of the unknown consists of a mixture of colloids while the standard is a starch solution. The adjustment of the volumes, or perhaps better still, the use of the

standard adjusted to varying heights, is the safer course. In the present paper the authors have avoided corrections because it is very probable that the same purpose can be accomplished by the use of a single standard set at a given point (30 mm.). Of course, there is a sacrifice to accuracy, but it is doubtful whether absolute accuracy is essential in experiments such as this, where the range is determined only by the limits of the nephelometer scale. It is well to remember that when hundreds of samples in a series are ready to read, the work must be completed within a few hours, otherwise serious errors will occur on account of the differences in time for diffusion between the first and the last members of each series. Where the filtered samples are held there is danger of coalescence.

THE EFFECT OF PHYSICAL INJURIES ON THE PROTOPLAST INDUCED BY ARTIFICIAL CURING, AS MEASURED BY THE QUANTITY OF LEACHED MATERIAL

Although some data have been presented which indicate that both the refractometer and nephelometer tests are delicate in the measurement of the quantity of material leached from seeds differently treated, nevertheless the authors have deemed this phase of the investigation sufficiently important to add a series of tests on viable, yet injured seeds. As previously stated, this study is the offshoot of an experiment to determine the heat resistance of immature sweet corn.

Country Gentleman sweet corn was picked at three different stages of maturity and dried in a specially constructed seed-corn drier provided with wire shelves at different heights so that three temperatures, 47°, 43°, and 37° C., called high, medium, and low, respectively, in Table 4, could be maintained. Circulation was provided by means of a forge blower, while the heating arrangements consisted of large electrical heating units located at the base of the drier.

TABLE 4.—*Effect of physical injuries caused by artificial curing on the quantity of material leached*

Temperature in drier	Time in drier (hours)	Initial moisture (per cent)	Greenhouse test			Refractive index	Colloidal index
			Mean germination (per cent)	Mean increases over controls			
				Green weight (gm.)	Height (mm.)		
High	48	69	3.8	−0.16	−63.8	1.33505	2.18
Medium	48	69	17.5	−0.19	−66.2	1.33514	4.09
Low	48	69	41.2	−0.19	−35.3	1.33476	6.60
High	96	69	0	−0.26	−112.2	1.33560	1.98
Medium	96	69	12.5	−0.25	−85.2	1.33491	3.09
Low	96	69	33.8	−0.22	−48.4	1.33482	5.56
Room		69	70.0	−0.02	11.1	1.33443	12.30
High	48	44.2	98.8	0.30	61.8	1.33410	27.72
Medium	48	44.2	100.0	0.18	53.6	1.33408	30.65
Low	48	44.2	93.8	0.28	52.0	1.33414	21.72
High	96	44.2	71.2	−0.01	−11.8	1.33442	9.72
Medium	96	44.2	98.8	0.26	66.0	1.33422	18.05
Low	96	44.2	83.8	0.21	45.7	1.33424	20.82
Room		44.2	97.5	0.66	92.7	1.33400	51.30

The initial moisture contents of the two pickings reported in Table 4 were 69 per cent and 44.2 per cent, respectively, computed on the dry basis. Duplicate readings were taken of duplicate samples from each ear. Thus the figures in Table 4 are the averages of eight readings. The samples were soaked at 30° C. for 48 hours, the proportions being 5 gm. of sweet corn in 50 c. c. of distilled water. The nephelometer standard was set at 30 mm. and the refractive indices were read at 21°.

In Table 4 the corn with 69 per cent initial moisture shows clearly that the quantity of leached material increases in relation to the degree of injury to the protoplast as affected by temperature and by the period of exposure. The injury done by such treatments is clearly manifested by the germination and growth of the seedlings and the refractive and colloidal indices. The latter, it will be noted, are in close agreement with the former. In cases where the initial percentage of moisture is 44.2 the agreement is not so close, but this is exactly what is to be expected in a case where the external factors have little or no injurious effect. The data in Table 4 show how remarkably sensitive the protoplast really is and that even slight changes in external conditions are sufficient to modify the permeability and the subsequent growth.

The growth records for the entire series have been correlated with the respective refractive and colloidal indices obtained from identical samples just previous to planting, and are given in Table 5. The frequency distribution in Table 6, which also gives the methods of calculation in detail, shows the close agreement between permeability and subsequent growth.

TABLE 5.—*Correlations between seedling characters and instrument readings for 116 ears of Country Gentleman sweet corn cured by artificial heat*

Seedling characters	Refractive index (21° C.)	Colloidal index (Standard 30 mm.)
Per cent germination	r_{xy}=0. 592±0. 041	r_{xy}=0. 634±0. 037
Difference in height as compared with control.	r_{xy}= . 360± . 055	r_{xy}= . 680± . 034
Difference in green weight as compared with control	r_{xy}= . 343± . 055	r_{xy}= . 693± . 033

TABLE 6.—*Correlation between the colloidal index and the difference in green weight of seedlings from sweet corn cured by artificial heat*

Colloidal indices	Green weights (gm.)														fy	dy	Σdx	$\Sigma dxdy$	fdy	fd^2y
	Less than control							More than control												
	0.60 .51	0.50 .41	0.40 .31	0.30 .21	0.20 .11	0.10 .01	0 09	0.10 .19	0.20 .29	0.30 .39	0.40 .49	0.50 .59	0.60 .69	0.70 .79						
1-3	2		3	15	7	3									30	0	94			
4-6	1	2	5	9	3	5	1	1							27	1	89	89	27	27
7-9	1			2	6	4		1	4	1		1			20	2	109	218	40	80
10-12					1			2	1	2	3				9	3	74	222	27	81
13-15						1	2	1	1		1		1		7	4	54	216	28	112
16-18							2	2							4	5	26	130	20	100
19-21						1	2					2			5	6	39	234	30	180
22-24									1						1	7	8	56	7	49
25-27									1	1					2	8	17	136	16	128
28-30									2						2	9	16	144	18	162
31-33									1	1	1				3	10	27	270	30	300
34-36														1	1	11	13	143	11	121
37-39											1		1		2	12	22	264	24	288
40-42										1					1	13	9	117	13	169
43-45															0	14				
46-48							1								1	15	6	90	15	225
49-51														1	1	16	13	208	16	256
x	4	2	8	26	17	14	8	7	11	6	6	3	2	2	116		616	2537	322	2278
dx	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Totals.					
fdx		2	16	78	68	70	48	49	88	54	60	33	24	26	616					
fd^2x		2	32	234	272	350	288	343	704	486	600	363	288	338	4300					

$$wx = \frac{616}{116} = 5.310 \qquad w^2y = 7.706 \qquad \frac{fd^2y}{n} - w^2y = 11.932 \qquad r_{xy} = \frac{7.131}{\sigma_x \sigma_y} = 0.69\text{r}$$

$$w^2x = 28.196 \qquad \frac{fd^2x}{n} - w^2x = 8.873 \qquad \sigma_y = \sqrt{11.932} = 3.454 \qquad \text{P. E.}_r = \pm 0.033$$

$$wy = \frac{322}{116} = 2.776 \qquad \sigma_x = \sqrt{8.873} = 2.979 \qquad \frac{\Sigma d_x d_y}{n} - w_x w_y = 7.131$$

In Table 5 the values of r indicate that there is a somewhat close3 degree of association between the colloidal index and the seedling behavior than in the case of the refractive index. This is to be expected for two reasons: (1) The content of soluble solids in seeds is relatively low and the Abbé instrument is not entirely reliable for readings in the fourth decimal place; and (2) since the colloids play by far the more important physiological rôle, the quantitative loss as measured by the nephelometer is a closer index of injury to the protoplast. This lack of entire agreement between the refractive and colloidal indices is brought out in Table 7, which has been calculated from a series of 500 ears not injured by heat. The correlation coefficient for this distribution is 0.713 ± 0.015, showing that there is a very significant tendency for the colloids to vary in relation to the total solids, but on account of the limitations of the Abbé instrument and the small precentages of soluble solids, the agreement between them is by no means perfect.

TABLE 7.—*Correlation between refractive and colloidal indices in a series of 500 ears of Country Gentleman sweet corn selected from the field*

Refractive indices	Colloidal indices																				f_y
	1–4	5–8	9–12	13–16	17–20	21–24	25–28	29–32	33–36	37–40	41–44	45–48	49–52	53–56	57–60	61–64	65–68	69–72	73–76	77–80	
1.3358		1																			1
1.3357																					0
1.3356																					0
1.3355																					0
1.3354		1	1																		2
1.3353		1																			1
1.3352	1		1																		2
1.3351	1	1		1																	3
1.3350	1	3	2	3	1		1														11
1.3349	3	6	5	3	4	2	1	2	1			2								1	30
1.3348		2	18	9	6	6	2	1	3		1										48
1.3347			2	4	14	9	2	3		1											35
1.3346				4	11	12	6	3	4	1	2		1				1				45
1.3345			2	2	10	7	14	12	13	5	5	4		1							75
1.3344			1	1	7	5	5	11	7	7	6	3	6	1	1		1		1	2	65
1.3343					2	6	4	8	17	15	3	5	9	1	5	1	1	1		1	79
1.3342						1	2	4	3	10	9	11	11	5	7	10	3		3	5	84
1.3341											3	1		1	2		2	2	1	5	17
1.3340																1				1	2
x	6	15	32	27	55	48	37	44	48	39	29	26	27	9	15	12	8	3	5	15	500

$r_{xy}=0.713\pm0.015$.

INFLUENCE OF THE STRUCTURE OF THE PERICARP ON PERMEABILITY

The question of the influence of the pericarp on the rate of diffusion of colloids naturally presented itself. Koritz [3] has measured the resistance of the pericarp of sweet corn to puncture. He found that there is no uniformity in resistance to puncture either among kernels on a single ear or between different ears in the same open-pollinated strain of sweet corn. This is to be expected on account of the hybrid nature of sweet corn. His investigations, however, lead to the conclusion that quite the opposite is true in F_3 self-pollinated, apparently homozygous lines. Here the resistance to puncture was uniform, both as to individual kernels on the ear and between different ears in the same strain. As Koritz and the authors collaborated by using the same samples in this series and made their respective observations at the same time, it is possible to compare the two sets of data.

The coefficient $r_{xy}=0.104\pm0.061$ is obtained when the penetration values of the 98 ears mentioned in Table 10 are correlated with the corresponding colloidal indices. This coefficient is less than twice the probable error and its significance is very slight. It was deemed advisable to confirm these observations with readings from pure line strains, especially in view of Hibbard's (*6*) observations that there is as much variation in behavior toward distilled water within pure lines as in a mixed progeny.

A further comparison was made between 68 ears selected from F_3 pure lines of Narrow Grain Evergreen sweet corn and 78 ears from F_3 pure lines of Country Gentleman sweet corn. Owing to the scarcity of available material it was impossible to secure sufficient ears from a

[3] KORITZ, L. A. A STUDY OF THE PHYSICAL PROPERTIES OF THE SWEET CORN PERICARP. [Unpublished thesis. Copy on file, Univ. Ill. Libr.]

single strain. Several strains were utilized and the penetration values with the respective colloidal indices of a few are given in Table 8. It will be noted that the penetration values are remarkably uniform, while the colloidal indices vary widely. These data indicate that the structure of the pericarp, as measured by a puncturing device, is not correlated with the rate of diffusion which varies independently. This is shown further by the coefficients of correlation between the colloidal indices and the respective penetration values. For the F_3 Narrow Grain Evergreen strains $r_{xy} = 0.177 \pm 0.056$ and for the F_3 Country Gentleman strains $r_{xy} = 0.022 \pm 0.039$. Neither of these coefficients has much significance.

TABLE 8.—*The relation between penetration values and colloidal indices in F_3 self-pollinated strains of sweet corn*

Strain number	Variety	Penetration value	Colloidal index	Strain number	Variety	Penetration value	Colloidal index
306-19	Country Gentleman	78.2	17.2	207-446	Narrow Grain Evergreen	43.9	37.5
		76.0	18.4			43.9	24.6
		76.0	12.4			47.7	30.0
		76.6	16.6			38.1	47.5
		76.0	40.2			47.7	21.2
		76.0	52.1			43.9	20.2
		77.7	19.4			47.7	38.6
334-82	Country Gentleman	46.4	52.2	248-493	Narrow Grain Evergreen	44.1	19.8
		45.3	37.9			41.2	32.0
		45.9	39.9			41.1	17.9
		47.5	28.3			41.2	37.2
		47.5	33.2			41.2	36.5
		47.9	51.2			46.0	6.8
		47.5	58.6			50.4	22.9
		47.7	27.1			41.1	32.8
		47.5	40.2			41.2	69.2
		47.5	15.2			43.0	26.6

The coefficients of correlation for the entire F_3 population have been summarized in Table 9. These coefficients are just as significant as in the case of the open-pollinated strains, indicating that permeability is just as variable. It is interesting to note that, as shown in Table 8, there is a tendency for certain strains to have a higher range of colloidal indices than others. This brings up the question of the relation of colloidal indices to disease susceptibility found in homozygous strains.

THE VALUE OF THE NEPHELOMETER TEST UNDER PRACTICAL CONDITIONS

For the purpose of further determining the reliability of the nephelometer test, a series of 98 ears was selected from a single open-pollinated strain of Country Gentleman sweet corn. These ears were classified according to the rag-doll test, but only two of the largest classes are given in Table 10. When the colloidal indices are compared with the respective rag-doll and greenhouse percentages of germination, it will be found that there is very close agreement. Occasionally an ear found good by the rag-doll test is pronounced poor by its low colloidal index. Greenhouse and field tests almost invariably support the quality as indicated by the nephelometer test of such an ear.

TABLE 9.—*Correlations between seedling behavior and colloidal indices in a population of F_3 inbred strains of two varieties of sweet corn*

Seedling character	Colloidal index of—	
	Narrow Grain Evergreen	Country Gentleman
Germination (greenhouse)	0.554±0.057	0.504±0.057
Difference in height as compared with control	.414± .067	.410± .064
Difference in green weight as compared with control	.408± .068	.444± .061

TABLE 10.—*Relation between seedling behavior and colloidal index in part of a 98-ear series of Country Gentleman sweet corn when rag-doll readings are constant (100 per cent)*

Ear No.	Colloidal index (standard 30 mm.)	Rag-doll test—seedlings diseased (per cent)	Greenhouse test			Ear No.	Colloidal index (standard 30 mm.)	Rag-doll test—seedlings diseased (per cent)	Greenhouse test		
			Germination (per cent)	Average increases over control					Germination (per cent)	Average increases over control	
				Height (mm.)	Green weight (gm.)					Height (mm.)	Green weight (gm.)
2	27.3	None.	95	−19.0	−0.37	11	51.0	None.	100	−42.1	−0.50
13	38.4	None.	100	−18.8	−0.47	12	26.1	None.	100	−22.4	−0.24
3	37.2	None.	100	9.0	0.03	4	30.6	None.	95	17.5	0.15
6	25.8	None.	100	32.5	0.17	15	32.4	None.	100	−23.0	−0.20
28	32.7	None.	100	−3.2	−0.16	8	26.1	None.	100	45.8	0.93
19	26.7	None.	90	−4.6	0.10	17	24.6	None.	95	35.0	0.67
21	9.3	None.	95	14.0	0.15	20	38.4	None.	95	9.8	0.36
1	30.3	None.	90	−57.2	−0.80	59	9.6	40	50	−167.1	−1.54
7	24.3	None.	100	29.7	0.56	60	6.0	40	70	−113.9	−1.10
24	42.0	None.	100	33.7	0.08	53	11.7	40	90	−32.3	−0.53
5	45.0	None.	100	34.7	0.47	57	3.3	40	60	−77.2	−0.62
14	40.5	None.	100	10.8	0.28	47	7.5	40	50	−118.3	−1.06
26	28.8	None.	100	27.2	0.14	56	6.3	40	70	−53.6	−0.35
27	75.6	None.	85	−35.4	−0.29	49	12.6	40	70	−75.1	−0.78
18	49.2	None.	95	2.6	0.12	54	1.5	40	35	−136.8	−1.00
22	56.1	None.	100	2.8	0.08	50	6.0	40	45	−121.4	−0.96
25	34.2	None.	100	31.8	0.07	55 *	18.0	40	70	−41.6	−0.25
9	36.6	None.	90	39.1	1.75	52	50.4	40	85	11.8	0.25
16	39.0	None.	100	18.3	0.50	58	4.2	40	65	−44.7	−0.18
23	38.4	None.	85	4.5	0.28	51 *	51.6	40	85	−4.2	−0.03
10	65.7	None.	100	52.4	1.85	48	15.6	40	60	−65.3	−0.33

* Filter very slowly.

NOTE.—Colloidal index readings from 10 gm. corn in 50 c. c. distilled water soaked at 30° C. for 72 hours.

It is of interest to note that samples taken from the 28 ears which show 100 per cent germination and are free from disease, as shown by rag-doll germination, are not in all cases in agreement with the nephelometer test. The colloidal index, 9.3, for ear No. 21, for example, indicates a poor quality ear; but by rag-doll and greenhouse tests it is of good quality. The opposite is true of ear No. 27. On the other hand, in the samples from ears which show 100 per cent germination and are 60 per cent disease-free, the agreement with the nephelometer test is much closer. The tendency for the green weights and heights of seedlings to agree more closely with the colloidal indices when the permeability of the protoplast has been increased through injury or disease, is one which the authors have frequently observed. The cause of this tendency is probably the earlier inhibition of measurable growth in cases where the injuries

due to disease are severe. If the weakness is slight, as in the case of the 28 ears which showed 100 per cent rag-doll germination and were free from disease, early seedling growth gives no visible evidence and there results an apparent disagreement with the colloidal indices. It is better to consider ears showing this discrepancy of questionable quality and to eliminate them. Further tests in the field will almost invariably show that such ears are susceptible to later injuries and disease. In some very favorable seasons they will do well, but under adverse conditions will rapidly deteriorate. Similar results were obtained by Burkholder (*4*) with beans. The same tendency has been observed on a much larger scale in connection with the tests covered by Table 16, which will be referred to in their place.

The data for the entire series of 98 ears are given as frequency distributions in Tables 11, 12, and 13. The respective coefficients of correlation have been calculated. In Table 11 the colloidal indices and percentages of greenhouse germination are in fairly close agreement, which confirms the observations made from Table 10. The coefficient of 0.498 ± 0.051 from Table 11 shows that a close degree of association exists.

In Table 12 the seedling heights and the colloidal indices are similarly compared, and from the coefficient of 0.534 ± 0.049 it is evident that the association is even closer, due to the fact that most of the seedlings are smaller than the controls. This shows further that in cases where the protoplasts are severely injured seedling growth will be in closer accordance with the colloidal index than it will in cases where the protoplasts are slightly injured.

Table 13 contains a comparison between the colloidal indices and the green weights of the seedlings from which a coefficient of 0.547 ± 0.048 is obtained. Here again there exists the same relationship as shown in Table 12, which further supports the conclusions already drawn.

TABLE 11.—*Correlation between the greenhouse germinations and colloidal indices in a single strain of Country Gentleman sweet corn*

Colloidal indices	Per cent germination																					f_y
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
1–4							2	1	1	1		1	1	1				1				9
5–8		2					1	1		1	3		3		2	1		1		2	1	18
9–12		1						1	1		1			1	2	2			2	1	3	15
13–16								1					1	1	1			1	2	1	5	13
17–20															4				1	1	3	9
21–24																					4	4
25–28																			1	2	4	7
29–32																			1	1	2	4
33–36																					2	2
37–40																		1	1	1	4	7
41–44																					1	1
45–48																					1	1
49–52																		2		1	1	4
53–56																					1	1
57–60															1							1
61–64																						
65–68																					1	1
69–72																						
73–76																		1				1
77–80																						
f_x	0	3	0	0	0	0	3	4	2	2	4	1	5	3	10	3	0	7	8	10	33	98

$r_{xy} = 0.498 \pm 0.051$

TABLE 12.—*Correlation between heights of seedlings and colloidal indices in a single strain of Country Gentleman sweet corn*

Colloidal indices	Heights of seedlings (mm.)														f_y
	Less than control											More than control			
	250–226	225–201	200–176	175–151	150–126	125–101	100–76	75–51	50–26	25–1	0–24	25–49	50–74		
1–4		1	1	2	2		1	1	1						9
5–8	1	1	1	4		5		1	3	2					18
9–12		1		2			5	1	3		1	2			15
13–16		1						5	1	5		1			13
17–20							1	1	3	1	2	1			9
21–24										1	1	2			4
25–28										4		3			7
29–32								1		1	1	1			4
33–36										1		1			2
37–40										1	5	1			7
41–44										1					1
45–48												1			1
49–52									1	1	2				4
53–56											1				1
57–60						1									1
61–64															
65–68														1	1
69–72															
73–76									1						1
77–80									1						1
fx	1	4	2	8	2	6	7	10	13	18	13	13	1		98

$r_{xy}=0.534\pm0.049$

TABLE 13.—*Correlation between green weights of seedlings and colloidal indices in a single strain of Country Gentleman sweet corn*

Colloidal indices	Green weights of seedlings (gm.)																				f_y
	Less than controls										More than controls										
	2.0–1.9	1.8–1.7	1.6–1.5	1.4–1.3	1.2–1.1	1.0–0.9	0.80–0.70	0.60–0.50	0.40–0.30	0.20–0.10	0.0–0.10	0.20–0.30	0.40–0.50	0.60–0.70	0.80–0.90	1.00–1.10	1.20–1.30	1.40–1.50	1.60–1.70	1.80–1.90	
1–4	1	1		2	1	1	1	1		1											9
5–8		1	4	2	2	2	2		2	1		1		1							18
9–12		1	1		1	1	5	3			2	3									15
13–16	1					1	2	1	1	4	2			1							13
17–20								2	1	3	2			1							9
21–24									1			2		1							4
25–28									1	2		1		1	1						7
29–32							1			1	1	1									4
33–36										1	1										2
37–40								1			1	2	2						1		7
41–44											1										1
45–48													1								1
49–52								1			2	1									4
53–56											1										1
57–60							1														1
61–64																					
65–68																				1	1
69–72																					
73–76									1												1
77–80																					
fx	2	3	5	4	4	5	12	9	7	13	12	11	3	5	1	0	0	0	1	1	98

$r_{xy}=0.547\pm0.048$

THE RELATIVE VALUE OF THE OPTICAL TESTS IN RELATION TO GERMINATION AND SEEDLING VIGOR

It will be noted that the correlation coefficients exhibit a considerable degree of variation. The significance of such variation may be explained, first, on the basis that the number of seeds used in making both growth and instrument readings is relatively small; second, on the basis that in the better seeds the dispersity of the colloids is extremely high and, consequently, for reasons which have already been discussed, the reliability of the readings on the nephelometer scale rapidly decreases. This lack of reliability where the dispersity is high in no wise affects the usefulness of this test, since before this condition is reached the seeds are of a superior class.

Further evidence in support of the nephelometer test is given in the frequency distribution as shown in Table 6. The variability of the colloidal indices increases in relation to the increases in green weight, and this is further strikingly supported by the behavior of the untreated series of ears in Table 13. Comparisons of the colloidal indices with germination as shown in Table 11, and with the heights of seedlings, as shown in Table 12, still further support the nephelometer test.

In order to determine precisely the extent of the variability in both the nephelometer and refractometer tests, the readings from two large series have been classified and the standard deviations (σ) calculated and presented in Tables 14 and 15. The values of σ for series 2, shown in Table 14, are given for both the rag-doll and greenhouse germinations. In this case the percentages represent readings from the same ears. No rag-doll records are given for series 1. It was impossible for the authors to secure enough ears in each 10 per cent class as here noted to give a reliable standard deviation. In cases where the number was not sufficient, the classes were omitted. A sufficient number of classes are represented, however, to show the trend distinctly.

The deviations for the colloidal indices in series 1 when arranged according to the percentages of greenhouse germination increase gradually up to 90 per cent. Beyond this point the deviation more than triples, which again shows that superior seed has a low colloidal leaching. This is not entirely supported by the distributions as shown in Tables 12 and 13. These tables show that a number of seeds with low colloidal indices gave high growth tests in the greenhouse. This apparent discrepancy between low colloidal index and high seedling growth vigor in the greenhouse, when followed in the field, vastly increases the claim of reliability of the nephelometer test. Field tests will show that seeds apparently vigorous in the germinator and in the greenhouse will fail to maintain this standard in the field. This is especially true when conditions are unfavorable.

In series 2 the trend is very similar, but more gradual. The rag-doll readings in series 2 show that the deviations in colloidal index become large while the germination is still extremely low, but this would be expected from the known unreliability of the rag-doll test.

TABLE 14.—*The standard deviations of the optical readings in relation to germination*

Germination (per cent)	Colloidal index of—		Refractive index of—	
	Series 1	Series 2	Series 1	Series 2
Greenhouse:				
0–10	1. 614±0. 140		0. 0006540±0. 0000600	
10–20	1. 896± . 404		. 0003162± . 0000533	
20–30	1. 416± . 390			0. 0001939±0. 0000414
30–40	2. 448± . 389		. 0001662± . 0000264	. 0001707± . 0000271
40–50				. 0001920± . 0000276
50–60				. 0001925± . 0000211
60–70		11. 412±1. 217	. 0000980± . 0000209	. 0002450± . 0000261
70–80	2. 616± . 394	12. 240± . 973	. 0001662± . 0000250	. 0001986± . 0000158
80–90	3. 444± . 411	14. 880± . 703	. 0001732± . 0000206	. 0002532± . 0000120
90–100	12. 528±1. 073	17. 760± . 502	. 0002456± . 0000210	. 0002149± . 0000607
Normal rag doll:				
0–10				
10–20				
20–30				. 0002724± . 0000392
30–40		6. 880± . 989		. 0003645± . 0000524
40–50		18. 620±2. 374		. 0002771± . 0000353
50–60		16. 772±1. 885		. 0002728± . 0000307
60–70		20. 952±1. 766		. 0002663± . 0000224
70–80		13. 906± . 926		. 0002161± . 0000143
80–90		16. 496± . 775		. 0002315± . 0000109
90–100		17. 224± . 530		. 0002256± . 0000069

TABLE 15.—*The standard deviations of the optical readings in relation to green weights and heights of seedlings*

	Colloidal index of Series 1	Refractive index of Series 1
Green weight (in grams) as compared with controls:		
−0.60 to −0.41	2. 064±0. 402	
−0.40 to −0.21	1. 818± . 149	0. 0007142±0. 0000584
−0.20 to −0.01	3. 987± . 342	. 0003958± . 0000339
0 to 0.19	5. 424± . 480	. 0002248± . 0000277
0.20 to 0.39	10. 731±1. 241	. 0001846± . 0000214
0.40 to 0.59	9. 810±1. 560	. 0001572± . 0000250
Height (in millimeters) as compared with controls:		
−140 to −151	1. 479± . 204	. 0004230± . 0000582
−100 to −61	1. 926± . 192	. 0007734± . 0000769
−60 to −21	2. 682± . 294	. 0003864± . 0000423
−20 to 19	10. 419±1. 205	. 0002916± . 0000337
20 to 59	7. 185± . 831	. 0001912± . 0000221
60 to 99	9. 300±1. 230	. 0001442± . 0000191

In Table 15 the standard deviations of the colloidal indices are arranged with reference to the green weights and seedling heights. In both cases deviations become larger as the vigor of the protoplast increases. There is some tendency for the deviations to decrease where growth is exceptionally vigorous, but this could not be confirmed on a larger scale because of the extreme scarcity of such ears.

When the coefficients of correlation for three different series are arranged in an ascending order with respect to their mean percentages of germination (Table 16), it will be found that the correlation coefficients for the colloidal indices decrease. This is due simply to the greater variability which is always found where the protoplasts are only slightly injured and emphasizes the limitations of seedling tests, whether rag-doll or greenhouse. Table 16 also confirms in a very decided way the data in Tables 14 and 15.

The corresponding deviations for the refractive index readings in series 1 and 2 are also compared in Tables 14 and 15. In series 1

there is some tendency for the deviations to decrease in relation to the increased vigor of the seedlings, but this is not sufficiently definite to be taken into consideration. In series 2, Table 14, the deviations vary but not in a definite direction. The authors believe that the variation in size of the standard deviation with respect to the refractive indices is largely due to experimental error. It is known that the soluble solids consist mainly of sugars and a small proportion of electrolytes. The total percentage is small and the range of refractive indices is, therefore, somewhat constricted, 1.3340 to 1.3360, with the larger part of the readings falling between 1.3343 and 1.3346. When this is coupled with the fact that the fourth decimal place is merely an approximation in the Abbé refractometer, it is evident that the standard deviations are largely a matter of chance.

This absence of greater variability at one end of the scale than at the other is a valuable characteristic, furnishing a check on the colloidal indices. The association between the two tests is close, as indicated by the high coefficient obtained from Table 7.

TABLE 16.—*The relation between the mean percentage of greenhouse germination and the nephelometer test*

Source	Greenhouse tests			
	Correlation coefficients			Mean germination (per cent)
	Germination (per cent)	Height	Green weight	
Table 5	0.634±0.037	0.680±0.034	0.693±0.033	55.8
Tables 11, 12, 13	.498± .051	.534± .049	.547± .048	76.5
500 ears, not previously given	.374± .026	.341± .027	.313± .027	86.5

SUMMARY

The permeability of the protoplast to colloids may be measured by their dispersity in distilled water leachings.

The healthy and vigorous protoplast allows only small quantities of colloidal materials to leach through its membranes.

Injury or disease may result in physical and chemical changes within the protoplast and in a weakening of the semipermeable properties of its membranes.

The vigor and subsequent growth of the seedling, aside from its genetic constitution, is determined by the state of the protoplast.

The total soluble solids in distilled water leachings as determined by the refractive index are likewise a measure of permeability. Because of the small percentage of total solids present and the limitations of the instrument this test is not quite as responsive as is the colloidal index.

The total leachings from the protoplast are in no wise related to the resistance of the pericarp to puncture.

Neither the rag-doll nor growing tests, whether conducted in the greenhouse or in the field, measure a weakened condition of the protoplast as conclusively as the optical tests.

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FACTORS INFLUENCING THE LOSS OF IODINE FROM IODIZED SALT[1]

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INTRODUCTION

The prevalence of goiter in certain sections of the United States has resulted in the general use of iodized salt for both man and animals. The State of Montana lies in one of these so-called "goiterous" regions, and in order to prevent goiter and its associated conditions of weakness in farm and ranch animals, the veterinary department has recommended the use of stock salt containing 1 ounce of potassium iodide to 100 pounds of the salt.[3] This salt is frequently prepared in large quantities and stored in convenient places on the range. After it has been in storage for several months or longer, stockmen have noted that brown specks appear scattered throughout the mass. The question was accordingly raised whether these brown specks were due to discoloration by iodine which had been freed from combination with the potassium. If the discoloration were due to iodine then some iodine would also escape into the atmosphere and the remaining salt would contain less of it. The study to be reported in this paper concerns itself with the loss of iodine from iodized salt, the factors which influence its loss, and methods for preventing it.

HISTORICAL

It is a common observation that solutions of potassium iodide become yellow or brown with age. The brown color of these solutions is due no doubt to the formation of free iodine resulting from the decomposition of potassium iodide. Concerning the mechanism or the dynamics of the change, little definite information is available. Exposure of the solution to sunlight apparently hastens the decomposition.

It is probable that the same factors which operate to liberate iodine from solutions of potassium iodide will operate in a similar way to produce free iodine in an iodized salt. Fellenberg (*5*)[4] finds that iodized salt in ordinary storage in a grocery store loses iodine. Since he found that the purer the salt the lower the loss of iodine, McClendon (*13*) suggests that the loss is probably due to the presence of nitrites or nitrates which oxidize the iodide to iodine, which then volatilizes into the air. Fellenberg (*5*) has determined the impurities likely to be present in salt. He found no correlation between the

[1] Received for publication Mar. 25, 1927; issued August, 1927. Published with the approval of the director of the station.

[2] The writers extend their thanks to Dr. Howard Welch, of the veterinary department, for his interest in this work and for the suggestions which he has made from time to time. It was he who originally suggested that the problem be investigated and who furnished some of the materials used in the investigation. The writers also desire to thank Dr. W. S. Bole for his kindness in permitting the use of his apparatus for producing ultra-violet and infra-red light.

[3] Since the beginning of these experiments, unpublished results of the veterinary department indicate that stock salts carrying as low as 0.02 per cent of KI are effective in preventing goiter.

[4] Reference is made by number (italic) to "Literature cited," p. 183 .

Mg, Ca, SO_4, Fe, and Al content of the salt and the quantity of iodine lost during storage.

Since iodine should not escape so readily from an alkaline medium, this same investigator (*5*) prepared an alkaline iodized salt by the addition of 0.693 gm. of potassium carbonate per kilogram of the salt. In several instances this alkaline salt retained more of its iodine than ordinary iodized salt when the two salts were stored under comparable conditions. Fellenberg (*5*) also investigated the distribution of iodine stored in containers of such a nature that he could obtain upper, middle, and lower layers of the salt. He found that in every case the iodine content was lowest in the middle layer and greatest in the lowest layer. This phenomenon he attributed to the solubility of the potassium iodide in the moisture of the salt and its transportation to the surface by capillarity. At the surface it concentrates owing to the drying out of the salt. Fellenberg also states that slightly moist salt retains its iodine better than dry salt and, further, that iodized salt loses its iodine as it drys out.

Fellenberg (*4*) determined the hydrogen-ion concentration of solutions of stored salt from time to time. If iodine were lost from the salt then the remaining salt should be more alkaline, due probably to the formation of potassium bicarbonate. Fellenberg, however, observed no significant changes in hydrogen-ion concentration.

Exposure to air and light are likely to affect the rate of loss of iodine from materials containing it. Several workers have detected iodine in the atmosphere. Chatin (*2*) was able to classify regions as goiterous or nongoiterous according to the iodine content of the air. Gautier (*11*) found appreciable quantities of iodine in sea air, less in air at Paris, and none in mountain air. The iodine which this last worker detected was all present as organic iodine. Fellenberg (*8*) has determined the influence of exposure to light and air on the loss of iodine from materials containing it. He stored sea water from two sources for periods of two to seven weeks in darkness and in daylight, in still air and in moving air. Only the sea waters which were exposed to a current of air appeared to lose any iodine. Whether stored in darkness or in daylight made no difference in the quantity of iodine lost.

Another means by which iodine could be liberated from materials containing it is by the action of molds or bacteria. This means is of special significance in the case of stock salts which may be scattered or kept in blocks on the ground or fed in troughs which become contaminated with soil.

Fellenberg, Geilinger, and Schweizer (*10*) found that certain agencies in the soil act on potassium iodide to liberate iodine. When a strip of moist starch paper was suspended in a beaker containing soil with added potassium iodide, it turned blue in a short time, indicating the liberation of iodine. The evolution of iodine into the atmosphere occurred more readily in unfertilized than in fertilized soils. This might indicate that the nitrites or nitrates of the soil were not responsible for the reaction. It is quite likely, however, that iodine was also liberated in the fertilized soils but reacted with and was bound by the organic matter.

Concerning the mechanism by which the iodine was liberated, Fellenberg, Geilinger, and Schweizer (*10*) state that the reaction was due neither to bacteria nor enzymes but to inorganic catalysts.

The ferric ion seems the most likely catalyst. Corresponding soils containing ferrous iron liberated some iodine, but the reaction was less rapid. In the case of soils of low iron content the liberation of iodine was more rapid at the higher hydrogen-ion concentrations.

Fellenberg and Geilinger (*9*) also found that *Bacillus coli*, *Aspergillus niger*, and various molds bound free iodine when cultured in neutral media. These microorganisms were not specific or active, but purely passive in their ability to react with free iodine. The iodine merely combined with the protein of the microorganism as it would with any nonliving protein.

EXPERIMENTAL

ANALYTICAL PROCEDURE

The best method of determining minute quantities of iodine which may be present in a material are those developed by Fellenberg (*4*, *6*, *7*) and by McClendon (*13*). The quantity of potassium iodide used in iodized salt, however, is sufficiently large so that other methods were found more convenient. The iodized salts on the market in this country contain several hundredths of a per cent of potassium iodide, while the stock salt recommended by the veterinary department of the Montana Experiment Station contains 0.0625 per cent.

For the determination of such quantities of iodine as these, a modification of Kendall's method (*12*) was found most suitable. According to this method the iodide is oxidized to iodate by means of bromine. The excess bromine is then expelled by boiling, the last traces being removed by treatment with salicylic acid. After acidification with H_3PO_4, potassium iodide is added and the liberated iodine titrated with standard thiosulphate solution. The thiosulphate solution used was approximately 0.01 normal. One cubic centimeter of such a solution is equivalent to 0.0002116 gm. of original iodine. Throughout all the work the thiosulphate solution was checked against a standard solution of potassium biniodate. This method may be used when large quantities of sodium chloride are present. When relatively small quantities of bromides are present, however, low results are obtained.

THE SALTS USED

The iodized salts were prepared from three types of commercial salts varying in size of particle. On the market these salts are known as table salt, hay salt, and stock salt. The salts were obtained from a salt manufacturer of Salt Lake City, Utah.

In the preliminary work attempts were made to prepare the iodized salts by adding potassium iodide to the salts in the form of a powder. Using this method it was found extremely difficult to obtain duplicate samples which would contain the same quantity of iodine. This was particularly true of the coarse stock salt. Since preliminary experiments indicated that some other method of preparing the iodized salt would have to be found, in order that uniform samples might be obtained, experiments were performed in which an aqueous solution of potassium iodide was added to the salt. It was thought that by this method the potassium iodide would be distrib-

uted more uniformly throughout the mass of salt and that on drying it would adhere to the salt particles. In any case the evaporation of the water would leave the potassium iodide more finely divided than by the previous method. By following this procedure it was possible to obtain more concordant results. It was accordingly adopted.

RESULTS

In giving the results which were obtained when the various iodized salts were stored under different conditions, each of several variables in the conditions of storage will be considered separately. Among the factors which were thought important as affecting the rate of loss of iodine from iodized salt were the moisture content as determined by the humidity of the surrounding atmosphere, the acidity or alkalinity which might be superimposed upon the salt, and the method of preparation of the salt, as, for example, whether the potassium iodide were added as a powder or crystallized from the brine with the sodium chloride. Salts iodized with potassium iodate were also prepared and stored under the same conditions as salts iodized with potassium iodide.

The work was also extended to include observations on the loss of iodine from iodized salt exposed to direct sunlight and to rain. The effect of heat alone on the loss of iodine was also studied.

Storage of Dry Iodized Salt in Pasteboard Cylinders

Samples of hay salt and stock salt were prepared, containing approximately the quantity of iodine recommended by the veterinary department. The original salts were moistened with a solution of potassium iodide, and then before storing in the covered pasteboard cylinders the iodized salts were dried by spreading out in a thin layer at room temperature. Periodically for a year the iodine contents of the salts were determined. The results are given in Table 1. A study of this table shows that no significant changes in the iodine content of the salts had taken place. Hence, it is safe to conclude that iodized salts kept in this manner will retain most of their iodine for storage periods of considerable length. Fellenberg (*5*) reached similar conclusions in regard to iodized salt prepared for human consumption and kept on the shelves of a store until sold.

Table 1.—*The loss of iodine from iodized salts stored in pasteboard cylinders*

Date	Storage period	KI content	
		Hay salt	Stock salt
	Weeks	*Per cent*	*Per cent*
Oct. 5, 1925	0	0.0565	0.0610
Nov. 28, 1925	8	.0546	.0612
Feb. 4, 1926	17	.0553	.0597
May 7, 1926	31	.0570	.0603
June 30, 1926	38	.0563	.0610
Sept. 5, 1926	48	.0572	.0623
Oct. 18, 1926	54	.0571	.0615

Storage of Iodized Salt Under Semisheltered Conditions

Samples of iodized stock salt were stored in such a way as to allow free circulation of air through them. One sample in an open glass jar was placed in an open shed. Another sample in a burlap bag was stored in the loft of a barn. The iodine contents of these salts were determined after various intervals of time. The results obtained are given in Table 2. These data indicate that considerable losses of iodine occurred under these conditions of storage. The salt stored in the burlap bag appeared to lose iodine more rapidly than the one stored in the glass jar. Thus, after about 13 months the salt stored in the burlap bag had lost 14.4 per cent of its iodine, while that stored in the glass jar had lost only 6.1 per cent. Since air would circulate much more freely in the salt stored in the burlap bag and remove the free iodine more rapidly with consequent shifting of the conditions back to where more iodine would be liberated, it is possible that this may account for the greater loss of iodine from the salt subjected to more aeration.

Table 2.—*Loss of iodine from samples of iodized salt stored under semisheltered conditions*

Stored in open jar in shed					Stored in burlap bag in barn loft				
Date	Storage period	KI	Loss per 10-gram sample	Loss	Date	Storage period	KI	Loss per 10-gram sample	Loss
	Weeks	*Per cent*	*Mgm.*	*Per cent*		*Weeks*	*Per cent*	*Mgm.*	*Per cent*
Sept. 29, 1925	0	0.0605	0	0	Oct. 26, 1925	0	0.0629	0	0
Nov. 30, 1925	9	.0606	0	0	May 20, 1926	29	.0589	.40	6.8
Feb. 3, 1926	19	.0594	.11	1.8	July 20, 1926	38	.0572	.57	10.0
June 25, 1926	39	.0590	.15	2.5	Nov. 22, 1926	56	.0550	.79	14.4
Sept. 10, 1926	50	.0575	.30	5.2					
Nov. 14, 1926	60	.0570	.35	6.1					

Storage of Iodized Salts in Atmospheres of Different Relative Humidities

Since iodized salts prepared on the range are likely to be stored under conditions differing in the relative humidity of the surrounding atmosphere, an experiment was devised to study this factor. The iodized salts were stored in desiccators in which the relative humidity was controlled by some substance or solution contained in the desiccator. The following relative humidities were maintained: 100, 50, 20, and 0 per cent. The 100 per cent relative humidity was maintained by having water in the desiccator, the 50 and 20 per cent by solutions of KOH, and the 0 per cent by solid $CaCl_2$. Potassium hydroxide was used for the intermediate relative humidities, as it would remove any free iodine from the atmosphere and hence would not allow the reaction by which iodine was liberated to stop or slow up because an equilibrium had been reached.

TABLE 3.—*Loss of iodine from iodized salts stored in atmospheres of different relative humidities*

Date	Storage period (weeks)	Percentage of KI at—			
		100 per cent relative humidity (over water)	50 per cent relative humidity (over KOH solution)	20 per cent relative humidity (over KOH solution)	0 per cent relative humidity (over $CaCl_2$)
Oct. 2, 1925	0	0.0630	0.0615	0.0688	0.0650
Nov. 27, 1925	8	.0563	.0621	.0644	.0637
Feb. 3, 1926	18		.0617	.0629	.0622
May 7, 1926	31	.0587	.0613	.0626	.0588
June 29, 1926	38		.0618	.0620	.0630
Sept. 3, 1926	48		.0615	.0620	.0635
Oct. 5, 1926	53		.0610	.0593	.0622
Feb. 8, 1927	71	.0517			

The data which were obtained are given in Table 3. These data indicate that iodized salt stored over water loses considerable iodine. After May 9, 1926, it was impossible to sample this salt as it had taken up so much water. On February 8, 1927, the whole mass of salt was dissolved in water and the iodine content determined. The salt stored in an atmosphere of 100 per cent relative humidity lost nearly 20 per cent of its iodine during storage from October, 1925, to February, 1927. The salt stored over the solution of KOH of such concentration as to give 50 per cent relative humidity lost no iodine during storage, while the two salts stored in drier atmospheres, i. e., over the more concentrated solution of KOH and over $CaCl_2$, lost small quantities of iodine. Further observations concerning the loss of iodine from very dry iodized salts will be given later. Fellenberg obtained similar data when he stored dry and moist iodized salts.

STORAGE OF ACID AND ALKALINE IODIZED SALTS

It is known that when free iodine is introduced into an alkaline solution it reacts with the alkali to form the iodide. On the other hand, iodine is readily liberated from acid solutions of the iodide. Hence iodized salts rendered alkaline may retain their iodine better than neutral or iodized salts rendered acid. In order to test the validity of this idea, one iodized salt was rendered acid by the addition of 1 per cent of KH_2PO_4 while another was rendered basic by the addition of 1 per cent of $NaHCO_3$. These salts were stored in a bell jar at ordinary humidity and the iodine content determined periodically. The results obtained are given in Table 4. The data in this table show that the salt rendered acid lost about one-third of its iodine during storage for aproximately one year. The salt rendered alkaline not only did not lose but actually showed an increase in iodine content when stored for a year. Since the two salts were stored under the same bell jar, the basic salt apparently absorbed a small quantity of the iodine liberated from the acid salt. That iodine was liberated from the acid salt and not from the basic salt was shown qualitatively. A piece of paper was placed in each salt. The paper placed in the acid salt became dark brown, while that in the basic salt showed no discoloration.

TABLE 4.—*The influence of added acid or alkaline salts on the loss of iodine from iodized salts*

Date	Storage period	KI content	
		Salt containing 1 per cent KH_2PO_4	Salt containing 1 per cent $NaHCO_3$
	Weeks	*Per cent*	*Per cent*
Oct. 2, 1925	0	0.0610	0.0622
Nov. 28, 1925	8	.0622	.0631
Feb. 4, 1926	18	.0509	.0630
May 7, 1926	31	.0468	.0640
June 28, 1926	38	.0451	.0645
Sept. 3, 1926	48	.0428	.0648
Oct. 13, 1926	54	.0410	.0649

The effect of the alkalinity in preventing loss of iodine from iodized salt is not in entire agreement with certain work of Fellenberg. He found that the addition of 0.693 gm. of K_2CO_3 to a kilogram of salt did not always prevent the loss of iodine, although in some cases it did. Fellenberg did not work with an acid salt. It is possible that a slight loss of iodine from the alkaline salt would have been found in the writers' experiments also if the atmosphere had not been so rich in iodine liberated from the salt which had been rendered acid. Data will be presented later, however, to show that the addition of $NaHCO_3$ distinctly reduces the loss of iodine from iodized salt.

POTASSIUM IODIDE LEACHED FROM IODIZED SALT BY RAINS

In order to study the effect of exposure to climatic conditions, particularly rain, on the loss of potassium iodide from salts containing it, a large deep funnel containing 2,240 gm. of the salt was set up out of doors. A bottle was placed under the funnel in order to catch the solution which would seep through the salt. It was thought that almost all the potassium iodide which was present in the salt would be removed by the first rain (on account of the great solubility of KI in water), and thereafter the residual salt would be practically iodine free. Such was not the case, however, as the data in Table 5 indicates. After several rains resulting in the collection of 667 gm. of leachings the salt still contained more than 20 per cent of its original potassium iodide. One would expect, moreover, to obtain more iodine in the first rains than in later rains. The rate of rainfall, however, appears to regulate this. The first rains were very hard, while those which came later were slower.

The reason that more potassium iodide is not removed is probably because the rain washes channels down through the salt and the channels once formed allow most of the water to pass through. The remainder of the salt was protected by air which was unable to escape through the wetted salt surrounding it. Hence the water does not come in contact with the potassium iodide in the body of the salt but only with that along the channel. The formation of these channels in the salt layer was checked up in the laboratory. Salt was placed in long tubes and water from a burette allowed to drip slowly on the salt. The effect of the water as it seeped through the tube was then noted.

TABLE 5.—*Potassium iodide leached from iodized salt by rain*

Date	Rainfall	Weight of leachings	Total solids in leachings	KI extracted	Percentage of original KI extracted
	Inches	*Grams*	*Per cent*	*Mgm.*	
July 29, 1925 [a]	0.31	114.0	14.0	109.0	8.5
Do		37.9	26.1	62.0	4.8
Aug. 3, 1925	.03				
Aug. 13, 1925	.01				
Aug. 14, 1925 [a]	.64	109.2	20.7	214.5	16.7
Aug. 15, 1925	.01	196.0		292.5	22.7
Aug. 20, 1925	.01				
Aug. 27, 1925	.38				
Aug. 28, 1925	.21	210.7	25.0	321.0	25.0
Total	1.60	667.8		999.0	77.7

[a] Collected in two portions.

At the end of the experiment the salt remaining in the funnel was dissolved in water, and the potassium iodide in an aliqout of solution was determined. The leachings contained 77.7 per cent and the residual salt 22.4 per cent of the original potassium iodide, respectively, making a total of 100.1 per cent. This indicates that all of the iodine is accounted for in the leachings and in the residue and that very little could have been liberated by sunlight or other climatic factors. The experiment was, however, too crude to enable one to make accurate observations concerning the effect of sunlight in liberating iodine from iodized salt.

THE EFFECT OF METHOD OF CRYSTALLIZATION ON THE LOSS OF IODINE FROM IODIZED SALT

In most of the study which has been recorded the iodized salts were prepared by moistening the sodium chloride with a solution containing the proper quantity of potassium iodide. It was thought that it might be possible to prepare iodized salt in which the potassium iodide would be either occluded or held in solid solution within the crystals of sodium chloride. If such were possible the rate of loss of iodine should be slower than when the potassium iodide is merely coated on the outside of the sodium chloride crystals, as is probably the case when the salts are iodized by adding a solution of potassium iodide. In following out this idea an iodized salt was dissolved in water and, by evaporation of the water, it was recrystallized. The salt was then dried and stored, in one instance, in a pasteboard carton and, in another instance, in a canvas bag. The same iodized salt but not recrystallized was stored in an open glass jar. The iodine content of the three salts was determined perodically. The results are given in Table 6. A study of the data in this table indicates that the recrystallized salt stored in the pasteboard carton lost no iodine during storage for a year. This is in agreement with the data in Table 1 in which several salts were stored in the same manner. A sample of the same recrystallized salt stored in a canvas bag lost about the same quantity of iodine (a slightly greater percentage) as did the iodized salt not recrystallized. Hence, it would appear that no advantage attends recrystallizing the salt in this manner.

TABLE 6.—*Comparison of losses of iodine from ordinary iodized salt and iodized salt recrystallized*

Recrystallized iodized salt stored in pasteboard carton			Recrystallized iodized salt stored in canvas bag			Iodized salt (not recrystallized) stored in open glass jar		
Date	Storage period	KI content	Date	Storage period	KI content	Date	Storage period	KI content
	Weeks	*Per cent*		*Weeks*	*Per cent*		*Weeks*	*Per cent*
Oct. 19, 1925	0	0.0794	Oct. 19, 1925	0	0.0798	Aug. 18, 1925	0	0.1205
Jan. 19, 1926	13	.0794	Dec. 21, 1925	9	.0762	Oct. 19, 1925	9	.1150
May 7, 1926	29	.0797	Feb. 3, 1926	15	.0751	Dec. 21, 1925	18	.1137
June 30, 1926	36	.0797	May 1, 1926	28	.0743	May 8, 1926	38	.1138
Sept. 7, 1926	46	.0792	June 30, 1926	36	.0720	June 30, 1926	45	.1130
Oct. 18, 1926	52	.0794	Sept. 3, 1926	46	.0720	Sept. 5, 1926	55	.1132
			Nov. 22, 1926	57	.0705	Nov. 1, 1926	63	.1125

In order to determine further whether mixed crystals of potassium iodide and sodium chloride were formed, the following experiment was performed: Seventy grams of iodized salt was dissolved in just sufficient water to dissolve it, and about half the water evaporated. The salt which separated out was then placed in a Büchner funnel and sucked dry. Then enough water was added so that a pasty mush was formed. This mush was stirred for a few minutes and finally the water was drawn off by suction. This process was repeated. If a solid solution of potassium iodide in sodium chloride had been formed during the recrystallization then the mother liquor as well as the crystals remaining on the filter should contain considerable quantities of potassium iodide. If a solid solution had not been formed then the potassium iodide should all be dissolved in the mother liquor due to its relatively greater solubility. The salt remaining on the filter was found to contain practically no potassium iodide, hence it may be concluded that mixed crystals had not been formed. Since the recrystallized salt used in the storage experiment had been prepared in this manner, there is no reason to expect that it would retain its iodine to any greater degree than salt iodized in the ordinary way. Since sodium chloride and potassium iodide are of similar crystal structure it might be possible to prepare mixed crystals containing the two salts if proper conditions were maintained during crystallization. The preparation of solid solutions of several of the alkali halides is recorded in the literature. Such mixed crystals of potassium iodide and sodium chloride would probably contain much more iodine than is present in iodized salt, and would have to be used for iodizing salt. There is the possibility that salts iodized with mixed crystals of potassium iodide and sodium chloride would lose their iodine at a slower rate than salt iodized with potassium iodide. In view of the relatively small losses of iodine from salts stored under even only moderately favorable conditions this possibility did not seem worthy of investigation.

THE EFFECT OF SUNLIGHT ON THE LOSS OF IODINE FROM IODIZED SALT

Since in the feeding of iodized salts to stock the salts are likely to be exposed to sunlight for various periods of time, and since it is known that sunlight hastens the decomposition of potassium iodide,

a study was made of the effect of sunlight on the rate of loss of iodine from iodized salts. The salts were stored in open crystallizing dishes on the sill of a south window and determinations of the iodine content were made periodically. Four salts were used in this study, (1) a salt iodized with finely divided KIO_3, (2) a salt iodized with KI solution, (3) and (4) two iodized salts which had been recrystallized according to the method used in the preceding section. The results obtained are given in Table 7. The most significant conclusion that can be drawn from these data is that salts iodized with KIO_3 retain their iodine when exposed to sunlight, while salts iodized with KI lose considerable portions of it. Thus, the salt which originally contained 0.0620 per cent of KI contained only 0.0165 per cent at the end of 63 weeks. The recrystallized salts show no advantage over ordinary iodized salts in regard to retaining their iodine when exposed to sunlight. Salt (4) was stored in a crystallizing dish of $4\frac{3}{8}$ inches diameter while the other salts were stored in dishes of $7\frac{1}{2}$ inches diameter. Hence, a smaller surface of the salt was exposed to the light. This probably accounts for the smaller loss of iodine in this case.

TABLE 7.—*Loss of iodine from several iodized salts stored on the sill of a south window. Salt (1) was iodized with KIO_3 powder; salt (2) with KI solution; salts (3) and (4) were iodized with KI and recrystallized*

Date	Storage period	KI content			
		Salt 1	Salt 2	Salt 3	Salt 4
	Weeks	*Per cent*	*Per cent*	*Per cent*	*Per cent*
July 30, 1925	0	0.0630	0.0620	0.0528	
Oct. 5, 1925	10	.0628	.0495	.0270	0.0780
Nov. 28, 1925	17	.0620	.0399	.0179	.0745
Feb. 4, 1926	27	.0613	.0355	.0173	.0729
May 7, 1926	40	.0627	.0299	.0072	.0694
June 28, 1926	48	.0630	.0280	.0065	.0663
Sept. 3, 1926	57	.0633	.0250	.0049	.0621
Oct. 13, 1926	63	.0636	.0165	.0040	.0588

Since the surface exposed to the light appeared to be a factor in determining the quantity of iodine lost from an iodized salt, this factor was subjected to further study. Ten gram samples of iodized salt were weighed out on watch glasses and some of the watch glasses containing the salt were placed in dark cupboards, others were placed on the roof in direct sunlight, and still others were placed on the roof in the sunlight but covered with other watch glasses. The data which were obtained are given in Table 8. The losses in iodine which occurred are relatively large. Thus, exposure of the salt for two days resulted in a loss of 40 per cent of the iodine. When the watch glass containing the salt was covered the loss was less, or only 14.9 per cent. In the experiment in which one day's exposure was allowed the sky was somewhat cloudy. This may account for its lower loss in proportion to the loss for two days. The losses in all cases, however, are relatively large.

TABLE 8.—*Effect of exposure to sunlight on the rate of loss of iodine from iodized salt*

Treatment	Exposure of a salt for 1 day	Exposure of another salt for 2 days
Control (stored in dark cupboard), per cent KI	0. 1662	0. 0530
Exposed to direct sunlight, per cent KI	. 1505	. 0316
Difference	. 0157	. 0214
Loss, per cent	9. 4500	40. 300
Control (stored in dark cupboard), per cent KI	. 1662	. 0530
Exposed to sunlight but kept under glass, per cent KI	. 1557	. 0451
Difference	. 0105	. 0079
Loss, per cent	6. 4900	14. 900

EXPOSURE OF IODIZED SALTS TO VARIOUS CONDITIONS OF TEMPERATURE AND LIGHT

In the data which have been recorded thus far it has developed that losses of iodine were prevented when the salts were iodized with KIO_3 and when salts iodized in the ordinary way had added to them 1 per cent of $NaHCO_3$. It would have been most desirable to expose such salts to direct sunlight in order to determine their efficiency in retaining iodine under such a condition. Such experimentation as was carried out in the preceding section was, however, very difficult, as in many cases the watch glasses containing the salts were upset by wind or rain and the whole experiment had to be repeated. In the experiments to be described in this section, artificial conditions of heat and light were therefore set up with the idea of paralleling as closely as possible natural conditions of exposure to sunlight. An attempt was also made to determine what wave lengths of light were responsible for the decomposition of potassium iodide. The results which were obtained, when several salts were exposed to varying degrees of heat and light, are given in Table 9. Salts A, B, and C were iodized with potassium iodide, C being the neutral salt, while A contained 1 per cent of added $NaHCO_3$ and B 1 per cent of KH_2PO_4. Salt D was a neutral salt iodized with KIO_3. Ten gram portions of each of the salts were weighed out into small evaporating dishes and exposed to the different degrees of light or heat.

TABLE 9.—*Exposure of several iodized salts to various conditions of light and heat. Salt A contained 1 per cent $NaHCO_3$; salt B, 1 per cent of KH_2PO_4; salt C was the neutral salt; and salt D was a neutral salt iodized with KIO_3*

Treatment	Percentage of KI in—			
	Salt A	Salt B	Salt C	Salt D
	Per cent	*Per cent*	*Per cent*	*Per cent*
None	0. 0568	0. 0561	0. 0569	0. 0600
Exposure to ultra-violet light for 10 minutes	. 0562	. 0550	. 0567	. 0595
Exposure to infra-red light for 30 minutes	. 0568	. 0551	. 0568	. 0595
Exposure to electric light for 70 hours	. 0551	. 0443	. 0520	----------
Exposure to electric light for 100 hours	. 0555	. 0405	. 0511	. 0603
Exposure to electric light for 167 hours	. 0550	. 0387	. 0497	----------
Exposure to heat on hot plate at about 80° C. for 70 hours	. 0543	. 0424	. 0395	. 0590
Exposure to heat on hot plate at about 80° C. for 77 hours	. 0556	. 0416	. 0394	. 0602
Exposure to electric light for 167 hours, last 67 hours on hot plate at about 80° C	. 0545	. 0346	. 0351	----------

In one instance the salts were exposed for 10 minutes to ultra-violet light generated by an apparatus used by a local physician. The salts were placed about 4 feet from the source of the light. A study of the data in Table 9 indicates that none of the salts lost significantly great quantities of iodine. The salt with the acid reaction, however, showed the greatest loss. It is likely that the time of exposure was not long enough to allow the decomposition to proceed very far. The apparatus was such, however, that the light could not be generated for longer periods of time without injury to the apparatus.

When portions of the same salts were exposed to infra-red rays (also produced by a physician's apparatus) for a period of 30 minutes at a distance of 3 feet from the source of light, only very slight losses of iodine occurred. It is significant, however, that again the salt rendered acid with KH_2PO_4 lost the greatest quantity of iodine. It is also probable in this case that exposure to the light for longer periods of time would have given more significant results, but again this was not possible.

Portions of salts A, B, C, and D were next exposed to the light from a 750-watt electric light bulb, the dishes containing the salts being placed 10 inches from the filament. One series was exposed for 70 hours, another for 100 hours, and a third for 167 hours. At the end of these periods of exposure the iodine contents were determined. The data show that the salt containing the iodate lost none of its iodine; salt A, rendered alkaline with $NaHCO_3$, lost very little; while the neutral and acid salts lost considerable quantities which increased as the time of exposure to the light was increased. Also the acid salt lost appreciably more than the neutral salt. This result might be expected as the instability of the iodides in acid solution is well known.

The effect of heat alone on the loss of iodine from the same series of salts was next investigated. The dishes containing the salts were placed on a hot plate with the heat turned "low." The temperature attained by the salt was found to be about 80° C. A study of the data in Table 9 shows that the salt iodized with iodate again lost none of its iodine and that the loss from the salt rendered alkaline was relatively slight. Salts B and C again lost considerable quantities of their iodine. It is interesting to note that in this experiment the neutral salt lost more of its iodine than the one rendered acid with KH_2PO_4. This behavior occurred in two instances when the salts were exposed to heat alone; hence it is not likely that it is due to experimental errors. The explanation for the phenomenon is at present unknown.

Salts A, B, and C were next exposed to heat and light simultaneously. Salt D was omitted from this experiment as it had already been shown to be stable when exposed to either heat or light alone. Salt A again showed itself to be very stable. It lost only slightly more iodine than when exposed to light alone for the same time. In every case the losses of iodine from the salt rendered alkaline with $NaHCO_3$ are slight. Salts B and C lost about 60 per cent of their iodine. The losses in the case of these two salts were greater than when they were exposed to light alone for the same period of time. Thus the iodine content of the neutral salt decreased from 0.0569 per cent to 0.0497 per cent when exposed to light alone, while when

kept at 80° C. for the last 67 hours of the 167-hour exposure to light it decreased further to 0.0351 per cent. The acid salt showed similar decreases from 0.0561 per cent to 0.0387 per cent and to 0.0346 per cent, respectively. From these data it is evident that both light and heat are factors affecting the rate of loss of iodine from iodized salt.

The experiments of Fellenberg (*5*) as well as some of those of the present writers, showed that iodized salts which were kept perfectly dry lost more iodine tkan moist salts. The factor of dryness and the effect of heat alone on the salt may be linked together in some way, as the salt kept at 80° C. on the hot plate was certainly dry.

Concerning the mechanism of the reactions which take place when iodine is liberated in solutions of potassium iodide or from iodized salt, little definite information is available. Since iodine is set free, the solution or the salt, as the case may be, should have an alkaline reaction due to an excess of base. To test out this idea, a solution of KI which had stood in the laboratory for several years and had become a dark brown was decolorized by the addition of finely divided silver. The silver reacted with the free iodine to form silver iodide, and when filtered the solution became water clear. The resultant solution was red to phenolphthalein and when titrated with standard acid it was found to be 0.013 normal in its alkalinity; with methyl orange the alkalinity was 0.016. Why such an alakline solution does not cause the combination of iodine and alkali to reform the iodide is not known unless the iodine exists in some complex combination, which is probably the case.

Fellenberg (*4*) attempted to measure the decomposition of iodized salt by determining the hydrogen-ion concentration of a solution of the original salt and then that of the salt after storage. An increase in alkalinity would indicate loss of iodine. He found that solutions of the salts prepared before and after storage showed no significant difference in hydrogen-ion concentration. The quantity of KI in the iodized salts which he used was, however, so low [5] as to render difficult the determination of any slight changes which may have taken place. In the writers' work it was found that whenever any appreciable loss of iodine occurred there was also a resulting change in hydrogen-ion concentration. Thus, at the end of the storage period when drops of indicators were placed upon small portions of the salt, the salt stored in an atmosphere of 100 per cent relative humidity (Table 3), which had lost a considerable quantity of iodine, was shown to be more alkaline than the same salt which was stored in an atmosphere of 50 per cent relative humidity.

This same fact was shown in another set of experiments. The hydrogen-ion concentrations of the series of salts listed in Table 9 as being exposed to the light of the 750-watt electric light bulb were determined before and after exposure to the light. The results are given in Table 10. A comparison of the values for iodine lost as given in Table 9 and changes in P_H as given in Table 10 shows the relation which exists. The salts which lost the most iodine show the greatest change in the hydrogen-ion concentration of their solutions. With the hydrogen electrode it was impossible to determine the hydrogen-ion concentration of solutions containing the iodate.

[5] The salts with which Fellenberg worked contained five parts of KI per million parts of the salt. This quantity of KI was in accordance with the recommendations of the Swiss Goiter Commission.

Colorimetric determinations indicated, however, that the acidity of the salt iodized with KIO_3 had not been changed by exposure to the light.

TABLE 10.—*The hydrogen-ion concentration of solutions of several iodized salts. Salt A contained 1 per cent of $NaHCO_3$; salt B, 1 per cent of KH_2PO_4; salt C was the neutral salt; salt D was a neutral salt iodized with KIO_3*

Salt	Hydrogen-ion concentration in terms of P_H [a]		Salt	Hydrogen-ion concentration in terms of P_H [a]	
	Original	Final		Original	Final
A	7.13	7.25	C	6.54	7.02
B	2.62	3.50	D		

[a] The hydrogen-ion concentrations were determined before and after the salts had been exposed to electric light for 100 hours.

RELATION BETWEEN THE QUANTITY OF IODINE PRESENT IN AN IODIZED SALT AND THE QUANTITY LOST

In order to determine whether a certain proportion of iodine was lost from an iodized salt or whether an absolute quantity of it was lost, two salts were prepared containing widely different percentages of iodine. These salts were stored and their iodine content determined periodically. The results which were obtained are given in Table 11. The data in this table indicate that a certain percentage of the iodine is lost and not an absolute quantity of it. Thus, during the same storage periods the sample containing originally 0.1205 per cent of KI lost 0.08 mgm. of KI per gram of salt, while that containing originally 0.0288 per cent lost 0.02 mgm. of iodide per gram of salt. Of KI lost, the sample containing 0.1205 per cent of KI lost 6.65 per cent, while that containing 0.0288 per cent lost 6.95 per cent.

TABLE 11.—*Relation of quantity of KI present in an iodized salt to that lost during storage*

Date	Iodized salt of high KI content		Iodized salt of low KI content	
	KI present	KI lost	KI present	KI lost
	Per cent	*Per cent*	*Per cent*	*Per cent*
Aug. 18, 1925	0.1205		0.0288	
Oct. 19, 1925	.1150	4.57	.0282	2.08
Dec. 21, 1925	.1137	5.65	.0280	2.78
May 8, 1926	.1138	5.57	.0281	2.43
June 30, 1926	.1130	6.23	.0274	4.85
Sept. 5, 1926	.1132	6.06	.0275	4.51
Nov. 1, 1926	.1125	6.65	.0268	6.95

Table 12 gives results from which similar conclusions may be drawn. In this case two series of salts containing different quantities of iodine were exposed to electric light for 100 hours. The quantity of iodine lost apparently depends on the quantity present.

TABLE 12.—*Relation of quantity of KI present in an iodized salt to that lost by exposure to electric light for 100 hours. Salt A contained 1 per cent of $NaHCO_3$; salt B, 1 per cent of KH_2PO_4; salt C was the neutral salt*

KI content	Salts of high KI content			Salts of low KI content		
	Salt A	Salt B	Salt C	Salt A	Salt B	Salt C
Initial KI content, per cent	0.0568	0.0561	0.0569	0.0114	0.0112	0.0114
Final KI content, per cent	.0555	.0405	.0511	.0106	.0084	.0095
Difference	.0013	.0156	.0058	.0008	.0028	.0019
Per cent loss	2.29	27.80	10.20	* 7.00	25.00	16.7

* A single determination, others in duplicate.

DISCUSSION

From the data which have been given it is evident that iodized salts show considerable variation in the degree to which they retain their iodine when stored for extended periods of time. It appears that when air is able to circulate freely over the salt or through it the loss of iodine is greater than when circulation of air is prevented. On this basis coarse-grained iodized salts should lose their iodine more rapidly than fine-grained salts. In several instances this has been found to be true. The data indicate quite clearly that when circulation of air is prevented, loss of iodine for all practical purposes does not occur even when the salt is stored for periods longer than a year, provided the salts are not too damp and provided they are not exposed to light of too great intensity. Salts may be stored in atmospheres up to at least 50 per cent relative humidity without serious loss of iodine. In fact, iodized salts stored in atmospheres of 50 per cent humidity appear to retain their iodine more effectively than salts stored in drier atmosphere.

Exposure of ordinary iodized salts to sunlight results in setting free very considerable quantities of the iodine which is present. In one instance 40 per cent of the iodine of an iodized salt was lost when a thin layer of the salt was exposed to direct sunlight for two days. This loss of iodine by the action of light may be reduced either by iodizing the salt with KIO_3 or by rendering it alkaline with 1 per cent of $NaHCO_3$. It is possible that a smaller quantity of $NaHCO_3$ might have been sufficient, but investigations with the addition of other quantities were not made in this study. Concerning the advisability of replacing KI with KIO_3 there may be a question. Crespolani (*3*) states that small quantities of KIO_3 are changed in the intestine to KI, and hence utilized in the same way as KI. The principal agency in the reduction which takes place is thought to be peptones in acid solution. He administered as much as 2 to 3 gm. at a dose. When such large doses were administered, however, some KIO_3 was excreted unchanged in the urine. Mellor (*14, p. 598*), on the other hand, states that care must be exercised in the choice of KI for medicinal purposes as it may contain some KIO_3, which, according to him, is a poison. It is not believed, however, that the small quantity of KIO_3 which would be taken internally in iodized salt would have any ill effects on the organism. In this regard McClendon in a private communication states that KIO_3 may be administered orally to human beings to the extent of ¼ gm. per

kilogram body weight. He advises against its injection into the system. Crespolani (*3*) also states that intravenous, intramuscular, or subcutaneous injection of iodates does not result in their reduction to iodides. From this he concludes, as has already been stated, that the reduction occurs in the alimentary canal. The use of KIO_3 to replace KI in iodized salt must, however, be a subject for further research.

According to Chapline and Talbot (*1*), the best way in which to feed salt to sheep is at the camp each night. Only what will be eaten is fed each time; hence there is no danger of losing iodine by exposure to sunlight. When, however, the salt is kept in a trough in the open sunlight for days or weeks at a time, considerable quantities of iodine will be lost before the salt has been eaten. The same is true of blocks or cubes of salt kept on the range. The iodine in the surface of the cubes may entirely disappear between the times when stock remove the outer surface. It would appear, therefore, that the use of iodized salt in blocks would not be a desirable practice.

At the beginning of the work it was reported that iodized salts containing brown spots were found, but no such spots have come under the observation of the writers. Van de Vorst (*15*) states that brown spots developed in postassium iodide due to the growth of a certain fungus. Such fungus growth might have caused the brown spots reported by the veterinary department.

CONCLUSIONS

Iodized salts stored in atmospheres of relative humidities of 50 per cent lose smaller quantities of their iodine than salts stored under similar conditions at other humidities.

Iodized salts rendered alkaline by the addition of $NaHCO_3$ lose practically none of their iodine during storage, while neutral salts or salts rendered acid lose appreciable quantities.

Salts iodized with KIO_3 lose none of their iodine when stored for extended periods.

Exposure of iodized salts to sunlight effects a loss of iodine from neutral or acid salts, only a slight loss from salt rendered alkaline, and practically no loss from salts iodized with KIO_3.

Exposure to heat alone effects losses of iodine from acid, neutral, and alkaline iodized salts and from salt iodized with KIO_3 in precisely the same order as did exposure to sunlight.

Exposure to light and heat simultaneously effects greater losses of iodine from salts of neutral or acid reaction than exposure to light alone or heat alone. In the case of iodized salts of alkaline reaction the losses of iodine are again insignificant or negligible, even though the salts are exposed to light and heat simultaneously.

The quantity of iodine liberated from a neutral salt iodized with KI appears to depend on the quantity of KI present.

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THE CURRENT MINERAL NUTRIENT CONTENT OF THE PLANT SOLUTION AS A POSSIBLE MEANS OF CHEMICAL CONTROL OF OPTIMUM FERTILIZATION [1]

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INTRODUCTION

During 1926 an attempt was made to estimate the current nutrient needs of various crops growing under various levels of fertilization. In order to secure such information determinations were made at intervals during the growing season of the nitrate nitrogen, phosphate phosphorus, and potassium contents of the plant solutions obtained from field crops growing in appropriate situations. In addition to these determinations, total analyses of the fertilizer ingredients were made on samples of tissue collected at the end of the growing season or, in a few cases, at various times during the season. In this paper the results obtained are given and the possibility of using the current levels of nutrient elements as bases for chemical control of fertilization is discussed.

ANALYTICAL METHODS AND CULTURAL CONDITIONS

The analytical methods employed with the plant solutions were those discussed by Gilbert in earlier papers.[2, 3]

The methods of analyses for the constituents of plant tissue used by the Association of Official Agricultural Chemists [4] were used for the total analyses in this study, with the exception that in the preparation of the samples for the determinations of P_2O_5 and K_2O a wet digestion with sulphuric and nitric acids was employed.

The fertilizer ingredients applied per acre are shown in Table 1. In each experiment, with the exception of the one in which nitrogen was used on the phosphate area, one fertilizer ingredient was definitely made suboptimal, past experience indicating that the quantities of the other fertilizers used were sufficient for the production of a normal crop. On the phosphate area the P_2O_5 was applied in acid phosphate; on the potash area the K_2O was applied in high-grade sulphate of potash; and on the market-garden area the nitrogen was applied in nitrate of soda, sulphate of ammonia, and tankage.

The growing season of 1926 seems to have been quite free from serious climatic growth-inhibiting conditions. Growth measurements on several crops and a chart of soil and air temperatures, accompanied by rainfall, are given in an earlier paper.[5] It was

[1] Received for publication May 2, 1927; issued August, 1927. Contribution No. 347 of the Rhode Island Agricultural Experiment Station.

[2] GILBERT, B. E. THE ADAPTATION OF CERTAIN COLORIMETRIC METHODS TO THE ESTIMATION OF NITRATES, PHOSPHATES, AND POTASSIUM IN PLANT SOLUTIONS. Plant Physiol. 1: 191–199. 1926.

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[5] GILBERT, B. E., MCLEAN, F. T., and ADAMS, W. L. Op. cit.

there reported that "the only noticeable depression in growth increments which may be attributed to temporary unfavorable conditions for growth, was during the period from July 15 to July 29." This was accompanied by a low rainfall and a high temperature period. The following statements were also made: "The moisture content of the soil was not critically low at any time during the summer. It fluctuated between 20 per cent and 35 per cent of the dry weight of the soil. The calculated wilting coefficient of the soil was about 12 per cent and the usually accepted optimum moisture content of the soil for plant growth was approximately 18 to 22 per cent." [6]

The highest yields of different varieties of crops investigated under different fertilizer treatments are given in Table 2. Subsequent tables give the relative yields for all plots based on the yields in Table 2, which are taken as 100. Due to the small areas planted and to the small number of plant individuals, the yields on the phosphate experiment were not expected to be of significance, the crops having been provided only for analyses.

TABLE 1.—*Fertilizer ingredients applied per acre to different plots*

Plot	N	P_2O_5	K_2O	Plot	N	P_2O_5	K_2O
	Pounds	*Pounds*	*Pounds*		*Pounds*	*Pounds*	*Pounds*
55N	[a] 55+40	50	135	116 MG (spring)[c]	0	200	30
65N	[a] 55+40	150	135	118 MG (spring)	100	200	100
658	[a] 80+60	150	135	119 MG (spring)	[d] 0+20	200	100
116	[b] 80+40	210	0	115 MG (fall)	[d] 70+40	120	90
117	[b] 80+40	210	50	116 MG (fall)	[d] 0+53	120	90
119	[b] 80+40	210	150	118 MG (fall)	[d] 70+20	120	90
115 MG (spring)[c]	60	200	30	119 MG (fall)	[d] 35+10	120	90

[a] Side dressings of $NaNO_3$ applied.
[b] Side dressings of cyanamid applied.
[c] These spring applications supplemented 16 tons of stable manure.
[d] Side dressings of ½ $NaNO_3$ and ½ $(NH_4)_2SO_4$ applied.

TABLE 2.—*Crops investigated and highest yields obtained from different varieties*

Crop	Area	Number of rows	Variety	Kind of tissue examined	Highest yield
					Pounds per row
Cabbage	Phosphate [a]	1	Enkhuizen	Trimmed heads	208
Do		1	Ballhead	do	129
Carrots		1	Danvers' Half Long	Roots	184
Turnips		1	Macomber Rutabaga	do	147
Buckwheat		2	Japanese	Green plants	40
					Cwt. per acre
Corn		5	R. I. White Cap	Grain and cob	67
Turnips	Potash [b]	8	Macomber	Roots	366
Parsnips		6	Champion Hollow Crown.	do	400
Potatoes		5	Rural Russet	Tubers	251
Tomatoes		6	Livingston's Globe	Fruit	482
Cabbage		8	Ballhead	Trimmed heads	355
Lettuce	Market garden [c]		Black Seeded Tennis Ball.	do	260
Celery			Golden Plume	Untrimmed heads.	339
Cabbage			Golden Acre	Trimmed heads	467
Beets			Early Wonder and Crosby's Egyptian (mixed).	Roots	224

[a] Rows were 96 feet in length.
[b] Rows were 30 feet in length.
[c] Crops covered whole plots (21 by 69.14 feet).
[6] GILBERT, B. E., MCLEAN, F. T., AND ADAMS, W. L. Op. cit.

CURRENT NITRATE-NITROGEN CONTENT OF PLANTS STUDIED

Four market-garden crops were selected for study. Of these, two were planted in the spring and two in the fall. Lettuce seed was planted April 16 and cabbage plants were set April 22. After these two crops were harvested beets were sown July 23 and celery plants were set July 19, on the respective areas.

In Table 3 the current nitrate nitrogen and the total nitrogen in tissue at time of harvest are shown, as well as the relative yields, and the quantity of nitrogen applied in chemical fertilizers. Each crop responded to the extra nitrogen application, and this extra quantity was reflected consistently in the plant solution throughout the season, the plants on the higher nitrogen plot having higher nitrate nitrogen in solution content. The total nitrogen results also correlate in general with these. The magnitude of the nitrate-nitrogen content is doubtless affected by side dressings of nitrogen made during the season. On May 27 an additional 20 pounds of nitrogen was applied to cabbages on plot 119. Celery on plots 115 and 116 received an extra application September 7, and beets on plots 118 and 119 were given 20 and 10 pounds, respectively, September 9. These applications are all reflected in the solution content of nitrate nitrogen.

The conditions on plots 65N and 65S of the phosphate area were studied. Among the miscellaneous crops, cabbage (Enkhuizen variety), carrots, buckwheat, and corn received attention. The cabbage plants were set June 15; carrot seed was sown May 11; buckwheat was sown May 14; and corn was sown May 10. Table 4 gives the current nitrate-nitrogen and total nitrogen contents of these crops, as well as the relative yields and the quantity of nitrogen applied in fertilizer. On July 16 side dressings of 40 and 60 pounds per acre of nitrogen were made to plots 65N and 65S, respectively. The effect of this application seems to be reflected in the cabbage solutions as analyzed July 26. The low rainfall and high temperature conditions just prior to this date probably helped to produce the abnormally high value of 600 parts per million of nitrate nitrogen in plant solution.

In the case of cabbage and carrot there is not such a clear-cut correlation between solution content and fertilization, as was noted with the market-garden crops. This could hardly be expected, as the nitrogen condition on plot 65N was not considered to be suboptimum. With none of the crops can the relative yields be considered as more than directional, and inconsistencies might be expected in the solution content. This is further proven by the lack of correlation between the total nitrogen in tissue at harvest and the relative yields. It would seem that under existing conditions of soil and climate, and with such a small difference in nitrogen application, little increase in yield or reflection in solution could be expected, since the nitrogen in both cases is present in sufficient amounts.

TABLE 3.—*Comparison of nitrate nitrogen in plant solution, and the quantity of nitrogen added in chemical fertilizer, market-garden experiment*

Crop	Plot	Tissue examined	Relative yield	N added in chemical fertilizer (pounds per acre)	Nitrate nitrogen in plant solution on certain dates (parts per million)						Total N in tissue on certain dates (per cent in dry matter)
					May 28	*June 17*	*June 25*	*July 9*	*July 23*	*July 30*	*July 19*
Cabbage (spring)	188MG	Lower leaves with midribs discarded	100	100	533	286	392	520	392	245	2.98
Do	119MG	do	81	20	233	130		272			2.92
					June 22	*July 8*					*July 9*
Lettuce (spring)	115MG	Leafy	100	[a] 60	417	454					4.83
Do	116MG	do	79	[a] 0	63						3.38
					Sept. 13	*Sept. 28*	*Oct. 1*	*Oct. 11*	*Oct. 18*		*Oct. 26*
Beets (fall)	118MG	Petioles and leaves	100	90	518	78	110	30	30		2.95
Do	119MG	do	72	45	106		42		30		2.81
					Sept. 8	*Sept. 20*	*Oct. 1*	*Oct. 5*	*Oct. 13*	*Oct. 14*	*Oct. 22*
Celery (fall)	115MG	do	100	110	517	340		308	500		3.20
Do	116MG	do	87	53	326	171	180			205	2.87

[a] 16 tons stable manure applied.

TABLE 4.—*Comparison of nitrate nitrogen in plant solution, and the quantity of nitrogen added in otherwise optimum chemical fertilizer, phosphate experiment*

Crop	Plot	Tissue examined	Relative yield	N added in chemical fertilizer (pounds per acre)	Nitrate nitrogen in plant solution on certain dates (parts per million)					Total N in tissue on certain dates (per cent in dry matter)
					July 15	*July 26*	*Aug. 19*	*Aug. 30*		*Aug. 24*
Cabbage (late)[a]	65N	Lower leaves with midribs discarded	83	95	209	600	340	248		3.44
Do	65S	do	100	140	200	600	358	365		3.41
					July 27	*Aug. 5*	*Aug. 18*	*Sept. 3*	*Sept. 16*	*Sept. 29*
Carrots	65N	Roots	100	95	100	137	69	71	76	1.36
Do	65S	do	87	140	78	318	91	169	31	1.73
					June 16	*June 28*				*July 15*
Buckwheat	65N	Stems and leaves	95	95	553	625				1.94
Do	65S	do	100	140	620	750				2.54
					July 16	*July 28*	*Aug. 9*			
Corn	65N	One foot of stem above upper node	98	95	319	288	79			
Do	65S	do	100	140	405	394	102			

[a] Enkhuizen variety.

TABLE 5.—*Comparison of potassium content of plant solution, and potassium oxide added in the chemical fertilizer, potash experiment*

Crop	Plot	Tissue examined	Relative yield	K_2O added in chemical fertilizer (pounds per acre)	Potassium content in plant solution on certain dates (parts per million)						Total K_2O in tissue on certain dates (per cent in dry matter)	
					July 9	*July 14*	*July 22*				*Aug. 10*	*Nov. 5*
Turnips	116	Leaves and petioles	13	0	1,047	919	1,394				1.04	
Do	117	do	71	50	2,807	1,956	2,398				1.52	
Do	119	do	100	150	3,140	2,899	4,253				1.52	
Do	117	Roots	71	50	1,944	2,002	2,556				2.31	3.21
Do	119	do	100	150	3,129	3,310	3,430				3.51	3.44
					July 2	*July 22*	*Aug. 2*	*Aug. 9*	*Aug. 24*	*Sept. 8*	*Aug. 16*	*Sept. 20*
Cabbage	116	Lower leaves with midribs discarded.	17	0	1,925		1,756	1,115	1,513	1,627	1.12	1.24
Do	117	do	60	50	2,326	2,773	2,227	2,066	1,686	2,011	1.72	1.62
Do	119	do	100	150	4,554	4,226	3,210		2,626	2,540	3.82	3.26
					July 28	*Aug. 10*	*Aug. 25*	*Sept. 9*			*Nov. 1*	
Parsnips	116	Roots	20	0	2,295	1,461	1,559	1,420			0.96	
Do	117	do	74	50	3,250	2,664	2,616	2,450			1.41	
Do	119	do	100	150	3,490	3,250	3,089	3,531				
					July 31	*Aug. 11*	*Aug. 23*	*Sept. 7*			*Nov. 2*	
Potatoes	116	Stem and tips	21	0	2,849	2,544	2,997					
Do	117	do	53	50	4,232	3,145	2,919	3,181			1.21	
Do	119	do	100	150	6,219	4,763	4,670	4,040			3.64	
					Aug. 5	*Aug. 10*	*Aug. 23*	*Sept. 7*	*Sept. 20*		*Oct. 11*	
Tomatoes	116	Stem and tips	35	0	2,728	2,796	2,054	1,897	1,620		0.38	
Do	117	do	45	50	3,129	4,020	3,083	2,363	1,834		.49	
Do	119	do	100	150	5,160	5,311	3,924	3,717	3,699		1.95	

CURRENT POTASSIUM CONTENT OF PLANTS STUDIED

Crops were planted on the potash experiment on the following dates: Turnips, seed, May 13; cabbage, plants, June 14; parsnips, seed, April 30; potatoes, tubers, May 3; tomatoes, plants, May 19.

In Table 5 the current potassium content of the plant solutions, the total K_2O in the tissue, the relative yields, and K_2O fertilization are given. It will be seen that the current amounts of potassium and the total K_2O in tissue quite consistently follow the fertilization, and that the relative yields correlate. As has been shown in an earlier paper,[7] the highest amounts of potassium in the plant solutions appear during the high temperature period, July 15 to July 29. This seems to explain the highest values of potassium which occurred with these crops.

CURRENT PHOSPHATE-PHOSPHORUS CONTENT OF PLANTS STUDIED

The planting dates of the crops analyzed on the phosphate experiment were: Turnips, seed, May 13; carrots, seed, May 11; corn, seed, May 10; cabbage (Ballhead), plants, June 22.

In Table 6 the current phosphate phosphorus and the total P_2O_5 found in the tissue during the season and at harvest, are shown, as well as the relative yields and the P_2O_5 fertilization.

It has been the experience of this station that with the same crops grown under these conditions, marked differences in yield, except with carrots, resulted between plots 55N and 65N. The general appearance of the above-ground portions of these crops confirmed such observations in 1926. This is reflected in the relative yields of corn and cabbage, but with turnips the relationship is not clearly shown. The current phosphate phosphorus seems to have followed the P_2O_5 applied, and the percentage of total P_2O_5 in the dry tissue was also consistent.

PLANT SOLUTION AS RELATED TO FERTILIZER APPLIED

From the foregoing it may be seen that the current amounts of nitrate nitrogen, potassium, and phosphate phosphorus in the plant solutions are influenced materially by the amounts of N, K_2O, and P_2O_5 supplied the crops in chemical fertilizers, and that, in general, these two amounts directly correlate. Some such relationship, although hardly as consistent, appears with total N, K_2O, and P_2O_5 in the dry tissue sampled both during growth and at harvest.

[7] GILBERT, B. E., MCLEAN, F. T., and ADAMS, W. L. Op. cit.

TABLE 6.—*Comparison of phosphate-phosphorus content of plant solution, and P_2O_5 added in chemical fertilizer, phosphate experiment*

Crop	Plot	Tissue examined	Relative yield	P_2O_5 added in chemical fertilizer (pounds per acre)	Phosphate-phosphorus content in plant solution on certain dates (parts per million)						Total P_2O_5 in tissue on certain dates (per cent in dry matter)	
					June 29	*July 20*	*Aug. 3*	*Aug. 16*	*Sept. 1*	*Sept. 14*	*Aug. 4*	*Oct. 18*
Turnips	55N	Roots	95	50	0.0	9.0	3.0	2.03	5.4	9.6	0.59	0.46
Do	65N	do	100	150	12.5	50.0	34.0	13.8	20.8	11.7	1.24	0.76
					July 27	*Aug. 5*	*Aug. 18*	*Sept. 3*	*Sept. 16*		*Sept. 29*	
Carrots	55N	do	97	50	25.0	13.4	18.0	17.0	11.7		0.66	
Do	65N	do	100	150	31.0	15.5	19.7	21.5	14.7		0.88	
					July 16	*July 28*						
Corn	55N	One foot of stem above upper node.	84	50	33.3	26.8						
Do	65N	do	98	150	38.4	28.8						
					Aug. 20	*Aug. 31*	*Sept. 15*				*Aug. 24*	*Oct. 4*
Cabbage (late) *	55N	Lower leaves with midribs discarded.	57	50	.86	4.45	1.38				0.45	0.43
Do	65N	do	100	150	1.11	5.20	1.56				0.79	0.73

* Ballhead variety.

DEGREE OF FLUCTUATION OF MINERAL NUTRIENTS IN PLANT SOLUTIONS

It is generally accepted that the relative metabolic needs of plants for the three mineral nutrient elements herein discussed are different. Hence it is to be expected that the plant solution would reflect this difference. This is the case. A comparison of the values given for each ingredient in Tables 3 to 6, shows that the standard deviations [8] of the ratios of the highest to the lowest values from the mean, are: Potassium, 0.84; phosphate phosphorus, 2.63; nitrate nitrogen, 4.09. Thus, the fluctuation of potassium during the growing season was smallest and of nitrate nitrogen greatest. This fluctuation must be considered as the resultant of the supply and demand of each ingredient; and as supply is regulated by soil, fertilizer applications, weather, and specific permeability of root membranes, it doubtless plays as great a part as do metabolic demands in determining the nutrient level in the plant solution.

THE PLANT SOLUTION AS A MEANS OF FERTILIZER CONTROL

It is suggested that the quantities of fertilizer nutrients as found at any one time may give some indication as to the needs of the plant for these particular nutrients. The individual nutrient contents in the plant solution have been shown to correlate with fertilizer applications, and the fluctuations of the nutrient elements have been considered to some extent. It may not be possible to choose arbitrary values, which may indicate critical nutrient situations, but from the data presented in this paper the following critical concentrations are tentatively suggested: Potassium, roots or tops, 3,000 parts per million; phosphate phosphorus, roots, 20 parts per million; nitrate nitrogen, blades of leaves, 300 parts per million.

It is recognized that these quantities can be only tentative at present, as it is not possible without further data to choose critical concentrations for individual crops, tissues, or soil situations. Further work is contemplated in this connection.

SUMMARY

In general the current concentrations of mineral nutrients in the solutions of crop plants were found to correlate directly with applications of chemical fertilizers.

Potassium was found to show the least fluctuation during the season, nitrate nitrogen fluctuated the most, while the fluctuation of phosphate phosphorus was intermediate.

The current mineral nutrient content of the plant solution is suggested as an index of fertilizer needs, and tentative critical concentrations have been chosen for each nutrient.

[8] In this calculation the highest value per ingredient, plot, and crop was divided by the lowest value, thus giving a ratio. These ratios were subjected to the standard deviation formula, i. e. $\sigma = \sqrt{\frac{\Sigma (f.\, d^2)}{n}}$.